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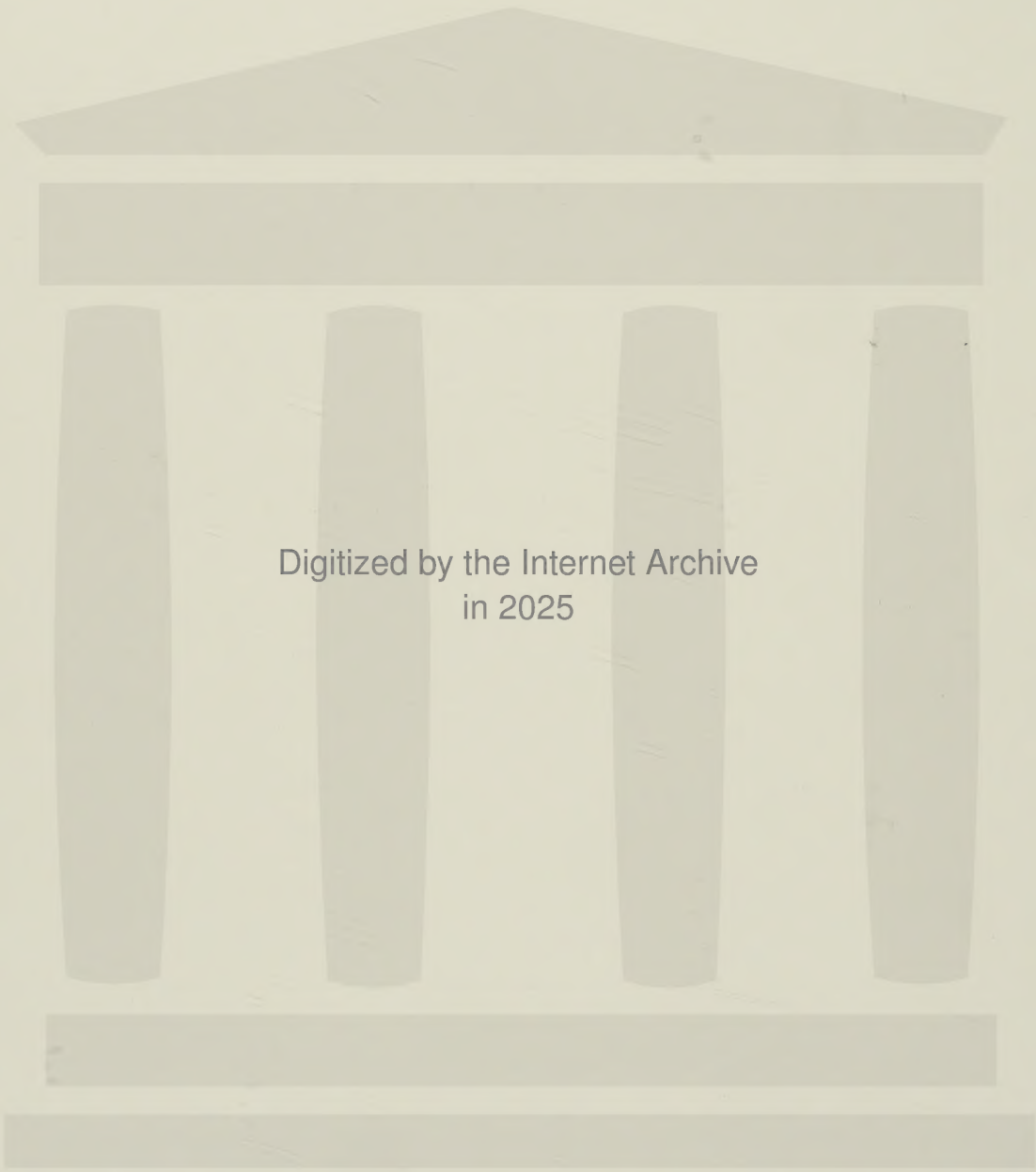
Papers presented at the

SEVENTH SESSION OF THE INDO-PACIFIC FISHERY COMMISSION WORKING PARTY ON FISH TECHNOLOGY AND MARKETING

Bangkok, Thailand, 19-22 April 1988

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS



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PREPARATION OF THIS DOCUMENT

The supplement to the report of the Seventh Session of the Indo-Pacific Fishery Commission (IPFC) Working Party on Fish Technology and Marketing contains the research contributions which were presented and discussed during the meeting. The editing was undertaken by R.A. Souness of the Department of Food Science and Technology, University of New South Wales, Australia. The references have not been checked for confirmity with the standard FAO format.

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ABSTRACT

The publication contains the thirty-seven research contributions which were presented as papers and discussed at the Seventh Session of the Indo-Pacific Fishery Commission (IPFC) Working Party on Fish Technology and Marketing held in Bangkok, Thailand, 19-22 April 1988. The papers have been divided into four categories: public health microbiology of cultured and wild shrimp; the occurrence of histamine in fishery products; salted and dried seafood products; miscellaneous section.

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EFFECT OF FARMING PHASE AND IN-PLANT PROCESSING ON THE MICROBIOLOGICAL QUALITY OF PRAWNS (Penaeus monodon)

by

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ABSTRACT

The bacteriological loads of farm-raised (brackishwater) prawns at the time of harvest and at different points during processing were determined. The total plate counts of freshly harvested prawns were between 6.8×10^4/g to 1.5×10^5/g. After processing, the counts decreased and ranged from 2.1×10^4 to 6.6×10^4/g. These counts are within the range of reported levels from temperate countries and are lower than the international standard of 10^6/g.

The use of animal (chicken) manure during the farming and fertilizing phase is a source of Salmonella contamination in prawns and should, therefore, be avoided. Proper handling and quality control immediately after harvest and during processing should result in high quality and safe products which would meet international standards.

1. INTRODUCTION

The demand for export trade in prawn/shrimp from the IndoPacific region and the Philippines in particular has been growing steadily. However, products are often seized, detained, rejected or destroyed for failure to meet importing country's standards of quality and safety. At present, the standards used are those set by the Codex Alimentarius Commission, the International Commission on Microbiological Standards for Foods (ICMSF), and the inspection services and public health agencies of both exporting and importing countries. These standards (Total Plate Count or TPC and presence of pathogens like Salmonella) were based from experiences and data in temperate countries because of lack of reliable data from tropical environments. It is possible then that the present levels selected may not be relevant or realistic to the tropical countries as evidenced by continuing incidences of rejection of prawn/shrimp by importing countries. Information is, therefore, needed on the initial counts (TPC) of prawn and the presence of pathogens at the time of harvest and while the prawns pass through the processing operation if exporting countries from the tropics are to influence international standards.

As part of the FAO collaborative study on prawn/shrimp hygiene, the following objectives were addressed:

1. To determine the bacteriological load of farm-raised prawn at the time of harvest and at different points during processing. Specifically,

   i) To determine whether prawns from tropical environments have significantly higher counts than those from temperate environments.
   ii) To determine the microbial quality of the head or gut trim and of the final product.

2. To determine the effects of farming/fertilizing phase (use of animal manure) on the bottom mud and on the natural microbial flora of the prawn. Specifically, to find out whether Salmonella is part of the natural microbial flora of the gut of prawn or is only present as a part of faecal contamination during the farming and fertilizing phase or during processing.

2. MATERIALS AND METHODS

The general work plan for the study, with the sampling points or areas indicated by (*), is shown below:

Process		Work Done
(*) Farming and fertilizing phase	-----	Impact on bottom mud
(*) Harvesting	----------	Initial microbial load of prawn
(*) Washing	----------	Effectiveness of washing at farm
Transport		
(*) Receival at plant	-------	Raw material quality
Removal of head/gut (beheading/ trimming) - (*) Gut trim	--------	Microbial status of gut trim (head)
Panning, packing		
(*) Finished product	--------	Microbial quality of final product

2.1 Sampling

Bottom mud samples from 4 sources (2 SEAFDEC experimental ponds, A and B and 2 commercial prawn farms, C and D) were collected the same day that the animal manure/fertilizer was applied. Sources A and B used mainly chicken manure; C used a mixture of chicken manure (500 kg), rice hull compost (300 kg), rice hull ash (200 kg), and commercial fertilizer (1 sack); and D, a mixture of chicken and carabao manure. Regardless of the area of the pond, a sample (6" x 6" x 4" thick) was obtained per 10 ft^2 area. Samples were analyzed for Total Plate Count (TPC).

2.2 Enterobacteriaceae and Salmonella

Prawn (Penaeus monodon) samples were obtained from a commercial processing plant (I) and from the same SEAFDEC experimental brackishwater ponds (II) where the bottom mud samples were taken. All samples were handled according to the flow diagram in Figure 1 except that Group II samples were not washed in chlorinated water (Step 5) but in chilled potable water.

2.3 Bacteriological Analysis

Bottom mud and prawn samples were tested for TPC and screened for Salmonella using the methodology outlined in Figure 2.

2.4 Total Plate Counts (TPC)

Trypticase Soy Agar (TSA) was used to enumerate TPC. The spread plate method was applied using 0.1 ml of the desired dilution. Duplicate plates were incubated at 25°C for 3 days and five out of every 50 samples were tested for TPC.

2.5 Enterobacteriaceae Counts

Enterobacteriaceae were enumerated on Violet Red Bile Agar (VRBA) using the spread plate method. Duplicate plates were overlayed with additional 10 mL of VRBA after 1 mL of the desired dilution was spread on the prepoured plate, then incubated at 37°C for 18-24 h. This procedure was used to screen samples for Salmonella. Samples with Enterobacteriaceae counts of 10^3/g or higher were selected for intensive salmonella testing.

2.6 Salmonella

Examination procedure for salmonella was done using the flow diagram in Figure 3. The Rappaport-Vassiliades (RV) (Vassiliadis et al. 1981) and US FDA (1976) methods were simultaneously used to test for the presence of salmonella.

3. RESULTS AND DISCUSSION

3.1 Bottom Mud Samples

The total plate counts (TPC) of bottom mud samples ranged from 4.4×10^4 to 6.4×10^7/g while the Enterobacteriaceae counts were from 1.7×10^3 to 4.8×10^4/g (Table 1). The percentage of Salmonella positive samples ranged from 0 to 23% (Table 1). The Enterobacteriaceae counts were utilized as a screening for further salmonella testing, with counts of 10^3/g or higher considered statistically likely to contain Salmonella . Results, however, showed that samples (B) with the highest incidence

of Salmonella (23%) had the lowest Enterobacteriaceae counts while samples with a nil incidence (D) of Salmonella had the highest Enterobacteriaceae counts (Table 1). Furthermore, Salmonella was detected in samples with less than 10^3/g Enterobacteriaceae counts, making the screening procedure unreliable. Table 1 also shows higher salmonella incidence in ponds utilizing either a chicken manure "cocktail" (C) or mixtures of chicken and carabao manure (D).

Table 1

Total plate counts (TPC), Enterobacteriaceae and Salmonella in bottom mud samples

Source	TPC (per g)	Enterobacteriaceae (per g)	Salmonella No. of positives	Salmonella Samples examined
A	6.4×10^7	9.6×10^3	3/20	(15%)
B	3.0×10^6	1.7×10^3	14/60	(23%)
C	4.4×10^4	4.4×10^4	1.51	(2%)
D	1.8×10^6	4.8×10^4	0/24	(0%)

The use of chicken and other animal manure is a source of bacterial and salmonella contamination. the animal manure/fertilizer used in ponds had very high tpcs (9.6×10^8 - 2.8×10^{10}/g) and while the Enterobacteriaceae counts varied (10^2 - 8.0×10^6/g), salmonella was consistently detected in all the 25 g samples as shown in Table 2. It should be noted that all the ponds used in this study were located near human settlements and in fact shared a common water supply (river) also where the settlements disposed of their sewage. While this study did not establish the effect of these settlements on the bacteriological quality of the ponds and prawns, sewage from the populace could also be a likely source of salmonella contamination.

Table 2

Total plate counts (TPC), Enterobacteriaceae and Salmonella in animal manure/fertiliser used in ponds

Source	TPC (per g)	Enterobacteriaceae (per g)	Salmonella in 25 g sample
A	2.9×10^9_8	8.0×10^6_2	Positive
B	9.6×10^8_9	$<10^2$	Positive
C	$4.8\times10^9_{10}$	3.6×10^6_3	Positive
D	2.8×10^{10}	$<10^3$	Positive

3.2 Prawn Samples

The TPCs of freshly harvested prawn ranged from 6.8×10^4 to 1.5×10^5/g (Table 3). These counts are within the range of reported values (10^3-10^5/g) of prawn/shrimp from temperate environments (Layrisse and Matches, 1984; Lillard et al., 1984; Matches and Layrisse, 1985) although Lannelongue et al. (1982) mentioned counts as high as 10^6 - 10^7/g. Counts reported from tropical countries also ranged from 10^3 to 10^6/g (Merican, 1984; Reilly et al., 1982; Surendran et al., 1985; Varma et al., 1982). A report by de Silva (1985), however, gave counts as high as 10^8/g. Results of this present study showed that freshly harvested tropical prawns can meet existing standards for TPC which is 10^6/g (ICMSF, 1974).

The effects of washing (W) and beheading (removal of head/gut) are also shown in Table 3. Samples collected from a commercial processing plant (I) had higher initial counts than those processed in the laboratory (II). Moreover counts from group I increased by 13% after washing whereas group II decreased by 43%. With proper washing (use of clean, potable, chilled water), the bacterial load of prawns can be reduced by as much as 65% (de Silva, 1985; Lisac, 1983; Rajaduraic,

1985). Prawns in group I were washed with water taken from the supply canal near the harvested ponds and while ice was added, the temperature was not low enough (10°C) which explains the higher counts after washing. In contrast, group II prawns were washed with potable, chilled water (4°C) at the pond site resulting in a 43% reduction in the bacterial counts. Transport from ponds to plant/laboratory resulted in significant increases (11-459%) of bacterial counts (Table 3). For both groups I and II samples, transport time was only 1 h, but while group II had reduced counts after washing and were properly iced (prawn: ice, 1:2), group I samples had additional contamination after washing and were not properly iced (prawn: ice, 1:<1).

Table 3

Total plate counts (per g) of prawns during harvest (H) and at different points during processing operation
(W = prawns washed at pond site;
R = raw material quality/prawns on arrival at plant;
T = gut trim or head;
F = final product quality)

Source	H	W	R	T	F
I	1.5×10^5	1.7×10^5	9.5×10^5	2.8×10^6	6.6×10^4
II	6.8×10^4	3.7×10^4	4.1×10^4	3.0×10^5	2.1×10^4

Beheading and washing procedures together can reduce bacterial load by as much as 90% (Layrisse and Matches, 1984). Table 3 shows reductions of bacterial counts from 30 to 48% after beheading and from 56-69% after washing and beheading. The TPCs of head/gut trim were between 3.0×10^5 to 2.8×10^6/g which were the highest counts compared to the rest of the prawn parts examined. Thus confirming that the concentration of bacterial load is in the head and gut portions.

Salmonella was not isolated from freshly harvested prawns and at any points during processing in group I (Table 4). The prawn samples were grown in ponds which were not fertilised with animal manure. Quality control was employed during the processing operation. Upon arrival at the plant, prawns were sorted and graded, washed twice in 2.5 ppm chlorinated water (27°C) then beheaded and trimmed. The chlorinated water solution used for washing was only used two or three times and immediately replenished. In and around the processing area, sanitation practices were also employed. The entrance to the plant has a depressed area which contained a lysol solution as a foot bath. A sanitary basin with a phisohex soap solution was provided at the exits of toilets. Processing tables were made of stainless steel which inhibits bacterial build up. The personnel used protective gowns/clothing, hair nets, gloves and rubber boots. Access to the plant was limited and the surrounding areas clean adn free of stray/roaming animals. Given the farming and processing operations employed by the plant, it is not surprising that in more than 10 years that it has been operational, it has never experienced rejection of its product.

Table 4

Incidence of Salmonella (samples positive/total no.examined) in prawn at harvest and at different pointsduring processing.

Source	H	W	R	T	F
I	0/50	0/50	0/50	0/50	0/50
II	4/120 (3.3%)	1/120 (0.8%)	0/120 (0%)	13/1200 (10.8%)	0/120 (0%)

Table 4 shows the incidence of salmonella in prawn in ponds fertilised with chicken manure (Group II). Salmonella was isolated in freshly harvested prawns (H, 3.3%), after they were washed at the pond site (W, 0.8%) and in the head/gut trim (T, 10.8%). Washing significantly reduced the salmonella load. The highest incidence was in the head which is consistent with the observation that the head/gut contain the bulk of bacterial load. The species of prawn (Penaeus monodon) used in this study bury themselves in the mud so that the gills and other parts of the head become highly contaminated.

Contrary to the assumption that prawns with Enterobacteriaceae counts of 10^5/g or higher are most likely to contain Salmonella, the organism was detected even in samples with less than 10^5/g counts (Table 5). Enterobacteriaceae counts followed the same trend as the TPCs during the processing operation.

It appears from these results that salmonella is not a part of the natural microbial flora of prawns but is present as part of contamination from animal manure and most likely from nearby human settlements.

Table 5

Enterobacteriaceae counts (per g) of prawns at harvest(H) and at different points during processing

Source	H	W	R	T	F
I	7.4×10^3	1.2×10^4	1.6×10^4	4.1×10^4	5.1×10^3
II	5.1×10^4	2.1×10^4	1.5×10^4	6.5×10^5	1.9×10^2

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STUDIES ON MICROBIOLOGY OF FARMED SHRIMP

by

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ABSTRACT

A problem besetting the shrimp industries is the implementation of microbiological standards for export. Studies on farmed brackish water shrimp aimed to reveal whether the microbiological load of tropical shrimps was significantly higher than those from temperate waters and whether salmonella is a natural or commensal contaminant of farmed shrimp.

Results showed that the microbial load of farmed shrimp samples varied between $0.2x10^5$ up to $3.7x10^6$ depending on the environmental conditions. The microbial load of the surrounding waters and the sediments varied between $1.2x10^5$ to $7.6x10^5$ to $2.3x10^8$ respectively. The coliform contents of farmed penaeids varied between 460-1,100 whereas the surrounding water and sediments were between $11x10^3$ and 123 or 1,100 respectively. Faecal coliforms were not detected for either the shrimp or the surrounding waters, but found in the range of 10-200 on the sediment samples.

Salmonella was not detected in either surrounding waters or shrimp samples but was frequently found in the sediments.

Treatments with chlorine solution and sodium bisulphite during processing gave significant reduction in the bacterial load of the shrimp.

During freezing, the total bacterial count of farmed shrimp was substantially reduced and faecal coliforms and Salmonella were not detected in the frozen products.

1. INTRODUCTION

After the world oil crisis a few years ago, the Indonesian Government launched a national campaign to promote exports of non-oil and gas commodities. With long coastal zones and availability of vast mangrove areas suitable for brackish water culture, Indonesia has the potential to develop brackish water shrimp culture. In fact the government has declared prawns as one of the prime non-oil and gas export commodities, and major efforts have been devoted to increase shrimp production through intensive and semi-intensive brackish water shrimp programs. This is reflected in the continuing increase in the number of brackishwater ponds (tambaks) for penaeid shrimp culture projects and also in the development of freshwater shrimp ponds for Macrobrachium rosenbergii production.

The continuous increase in shrimp production undoubtedly requires a parallel improvement in marketing as well as quality control programmes. One of the problems besetting the shrimp industry is the implementation of a stringent microbiological standard for shrimp exports.

The Codex Alimentarius Commission and the International Commission on Microbiological Specifications for Foods (ICMSF) have set a standard either for raw or cooked shrimp. Standards have also been set by inspection services, and in Indonesia by the National Quality Control, Department of Trade and public health bodies.

In general, the microbiological levels selected as the basis for shrimp standards are drawn from experience in temperate countries. Consequently, some tropical shrimp exporting countries experienced great difficulties with the international quality standards. The difficulties in meeting those standards are evident from the high level of rejection of shrimp exports by the importing countries.

There are indications that tropical shrimp have higher bacterial load compared to temperate species (Peranginangin, 1982). Cold water shrimp frequently have bacterial loads of $10-10^3/g$,

although the range sometimes vary greatly and on certain occasions has been reported up to 10^7/g (Liston, 1980). In most cases, warm water shrimp and prawns have counts of 10^6/g on capture (Cann, 1974; Vanderzant et al., 1970).

Several workers reported that bacterial populations of aquatic species are predominantly affected by the environmental conditions rather than by species type. It is known that Penaidae are scavenger omnivores and feed on detritus. Moriarty (1975) emphasized the importance of bacteria in organic detritus as the food source of penaeids and found that 20-35% of organic carbon in the proventiculus of Metapenaeus bennettae feeding on muddy estuary sediment contaminated by bacteria. Naturally, when shrimp are cultured in water polluted by domestic sewage, they will contain some enteric and faecal bacteria, such as Salmonella and E. coli.

There is also a controversial discussion concerning the possibility that salmonella is intrinsically present in the gut of shrimp at catch, and that this organism is present in the product as a result of faecal contamination during processing.

Therefore, the importance of collecting data on initial bacterial flora and its variation while the shrimp pass through the various processing operations, cannot be over emphasized if the tropical shrimp producing countries are to comply with national and international standards.

2. OBJECTIVES

The study aimed to answer the following questions:

a. Is the microbiological load of tropical shrimp significantly higher than temperate water species?
b. Is Salmonella sp. a natural or commensal contaminant of shrimp in the same way as it is of cattle and poultry?
c. What is the impact of the process workers on product hygiene, and can effective hand washing be implemented?

3. MATERIALS AND METHODS

3.1 Materials

- Cultured brackish water penaeid shrimp (Penaeus monodon)
- Microbiological media
- Crushed block ice
- Insulated boxes

3.2 Methods

Shrimps were procured from brackishwater ponds at Karawang, West Java. Samples of shrimp, pond water and pond sediment were taken to RIFT laboratory, Jakarta, for analysis.

Samples of shrimp were collected at every step of the freezing process, as well as water, pan and ice samples used in the processing plants. Samples were collected in polyethylene bags and chilled in crushed block ice in insulated boxes.

Microbiological analysis consisted of examination for Enterobacteriaceae, total bacterial count and Salmonella sp. were then carried out. The bacterial count was determined using Nutrient Agar, while the Enterobacteriaceae and Salmonella sp. were examined through the procedures shown in Figures 1 and 2.

4. RESULTS AND DISCUSSION

4.1 Chemical Composition of Farmed Penaeid

Proximate analysis of farmed P. monodon is given in Table 1.

Table 1

Proximate composition of tiger prawn (P. monodon)

Composition	Whole shrimp	Headless shrimp
Moisture (%)	78.03	78.55
Protein (%)	18.28	18.39
Fat (%)	0.90	0.86
Ash (%)	1.56	1.12

During ice storage, pH of shrimp fluctuated although there was a tendency to decline in the first 5 days, but gradually increased in the subsequent days of storage.

TVB-N of farmed tiger prawn varied between 7 and 28 mg% N, whereas those dipped in bisulphite and chlorine ranged between 9 and 30 mg% N and 10 and 27 mg% N respectively.

The ammonia content was relatively low during the first 8 days of ice storage (0.005 to 0.025 mg%), but rapidly increased in the later stage up to 0.030 mg%. There was an indication that dipping in bisulphite and chlorine solutions would not significantly affect the ammonia content of the shrimp.

4.2 Microbiological Analysis

Results of microbiological analysis of samples collected from Cemara village brackishwater ponds, Karawang, West Java are shown in Table 2.

The results indicated that except for sediment and prawn samples taken from pond 2 (SP2 and PP2) Salmonella was not detected except in a single sample. The presence of salmonella was also accompanied by high coliform and E. coli counts. Low counts of coliform and faecal coliform in the other ponds indicated that the majority of the ponds in this area have not been polluted by domestic sewage.

Table 2

Microbiological analysis of water, sediments and prawn samples of Karawang brackish water ponds

Sample Code	Staphyl. ($x10^2$)	Salmonella	Coliform (MPN)	Faecal coliform	TPC ($x10^5$)
Water					
WP1	0.2	n.d.	210	n.d.	79-219
WP2	23.4	n.d.	1100	n.d.	296-756
WP3	n.d.	n.d.	n.d.	n.d.	0.2-0.3
WP4	28.0	n.d.	9.1	n.d.	4-17
WP5	n.d.	n.d.	3.6	n.d.	0.3-0.9
Sediments					
SP1	80-166	n.d.	160	100	608-658
SP2	2520-5400	+ve	1100	200	1792-2248
SP3	63-68	n.d.	34	10	4-53
SP4	189-208	n.d.	290	100	648-790
SP5	68-71	n.d.	120	100	55-90
Prawn					
PP1	210-280	n.d.	1100	n.d.	3.4-4.7
PP2	1610-2300	+ve	1100	10	5.2-23.3
PP3	30-90	n.d.	460-1100	n.d.	0.2-0.7
PP4	970-1260	n.d.	1100	n.d.	16.2-36.8
PP5	110-390	n.d.	1100	n.d.	6.6-12.6

The bacterial load of P. monodon harvested from brackishwater ponds of Tanjung Bungin, Bekasi, West Java (SP1)gradually declined during 5 days icing, but then rapidly increased to over 10^6/g after 8 days (Figure 3). Apparently the decline in the bacterial load during 5 days of icing was primarily due to the reduction of mesophilic bacteria, followed by the rapid growth of psychrophiles. As reported by previous workers, Bacillus, Micrococcus and Flavobacterium initially predominate immediately after icing, but are subsequently replaced by Achromobacter and Pseudomonas after 16 days icing (Campbell and Williams, 1952).

It is obvious from Figure 3 that treatments with chlorine and bisulphite solutions significantly reduced the bacterial load of whole shrimp.

Hydrogen sulphide producing bacteria varied between 10^2/g to 10^4/g. According to Barile et al. (1985), hydrogen sulphide producing bacteria are very resistant to chilling and consequently play an important role in the spoilage process of high protein foods stored at low temperatures.

During chilled storage experiments, staphylococci did not exceed a 10^3/g, whereas salmonella was not detected. Although the coliform count was as high as 2.4×10^2/g, but E. coli was not detected throughout the experiments.

Studies on microbiology of farmed P. merguiensis harvested from Kalibaru brackishwater pond, Jakarta, indicated that the bacterial load of whole shrimp was higher compared to headless shrimp. Whole P. merguiensis dipped in clean water for 30 seconds had a bacterial load of log 6.38, whereas headless shrimp treated in the same manner contained bacteria of log 5.65. Dipping in 3,000 ppm bisulphite solution for 5 minutes could reduce the bacterial load of headless shrimp down to log 4.8, and further reduced to log 4.4 after dipping for 10 minutes.

Hydrogen sulphide producing bacteria in whole P. merguiensis was found to be higher than headless samples. Dipping in a 2,000 ppm bisulphite solution for 5 to 10 minutes could eliminate hydrogen sulphide producing bacteria.

Dipping in a bisulphite solution also significantly reduced the coliforms and staphylococci contents of P. merguiensis. Treatment with 3,000 ppm bisulphite for 10 minutes significantly reduced the coliform and staphylococci contents to 9 MPN/g and log 3.1 respectively.

4.3 Isolation and Identification of the Bacteria

Isolation and identification of bacterial isolates were subjected to further tests to determine whether the isolates belonged to the genera of salmonella, or other species. All suspected isolates from waters, sediments and prawn were subject to this identification.

The microflora of the samples collected from brackish water ponds of Karawang and its vicinity is given in table 3.

Table 3

Identification of isolates from brackishwater pond samples of Karawang, W. Java and its vicinity

Code	Source	Species
ST4X	Sediment	Proteus mirabilis
ST5X	Sediment	Enterobacter aerogenes
S1	Sediment	Citrobacter freundii
S8	Sediment	Enterobacter aerogenes
T3	Brackishwater	Citrobacter freundii
ST4	Sediment	Proteus mirabilis
ST11	Sediment	Klebsiella sp.
ST19	Sediment	Proteus mirabilis
G9	Tiger Prawn	Enterobacter aerogenes
G10	Tiger Prawn	Enterobacter aerogenes
G3S	Tiger Prawn	Salmonella sp.
G4S	Tiger Prawn	Klebsiella oxytaca
1GS	Brackishwater	Enterobacter aerogenes
2S4	Brackishwater	Proteus mirabilis
3ST	Sediment	Salmonella sp.
3S4	Sediment	Pseudomonas sp.
GT23	Sediment	Proteus mirabilis
GT30	Sediment	Proteus mirabilis
GT39	Sediment	Proteus mirabilis

4.4 Changes of Bacterial Load During Processing

The bacterial load of processed shrimp is dependent on the method of processing as well as environmental factors of the processing plant, especially the quality of water and ice used during processing steps.

The bacterial load of ice and water used during processing is given in Table 4.

Table 4

Bacterial load of ice and water (log)

Location	Water	Water glaze	Ice
Brackishwater pond Karawang, W. Java	4.12-4.91	-	4.62-5.25
Processing plant Jakarta	2.45-3.81	3.58-4.36	5.14-5.23

The total bacterial load of shrimp samples gradually declined during processing. A significant drop in bacterial load was noticed when the shrimp arrived in the processing plant. Apparently, such a significant decrease was due to the rapid mortality of mesophilic bacteria caused by proper icing of the samples. The initial load of bacteria when the prawn samples arrived in the processing plant was log 6.99 to log 7.55, whereas the frozen shrimp product was between log 5.98 to log 6.73.

The E. coli count of shrimp samples also declined gradually during processing. However, E. coli was not detected in the final frozen shrimp samples. Likewise staphylococcus count also declined during processing, although it was also noticed that the water used for washing the shrimp in the processing plant was contaminated by staphylococcus as high as log 0.88. On the other hand, salmonella and Vibrio parahaemolyticus were not detected throughout the processing lines, although Rosmawaty et al. (1985) found the presence of salmonella on the frozen marine tiger shrimp immediately after processing. The absence of salmonella in the frozen farmed tiger shrimp might have also been caused by the absence of this bacteria in the raw material rather than the effect of processing measures.

5. CONCLUSION

Generally, farmed tropical penaeid shrimp from Indonesia have higher microbiological load compared to temperate water species, although the microbial counts and the presence of faecal coliform and salmonella varies from place to place depending on the environmental conditions of the ponds.

Salmonella is apparently carried by farmed shrimp especially if they are cultured in polluted ponds, but might also be caused by contamination during processing.

The adoption of appropriate sanitation and hygiene principles during shrimp processing could significantly improve the microbiological quality of frozen shrimps.

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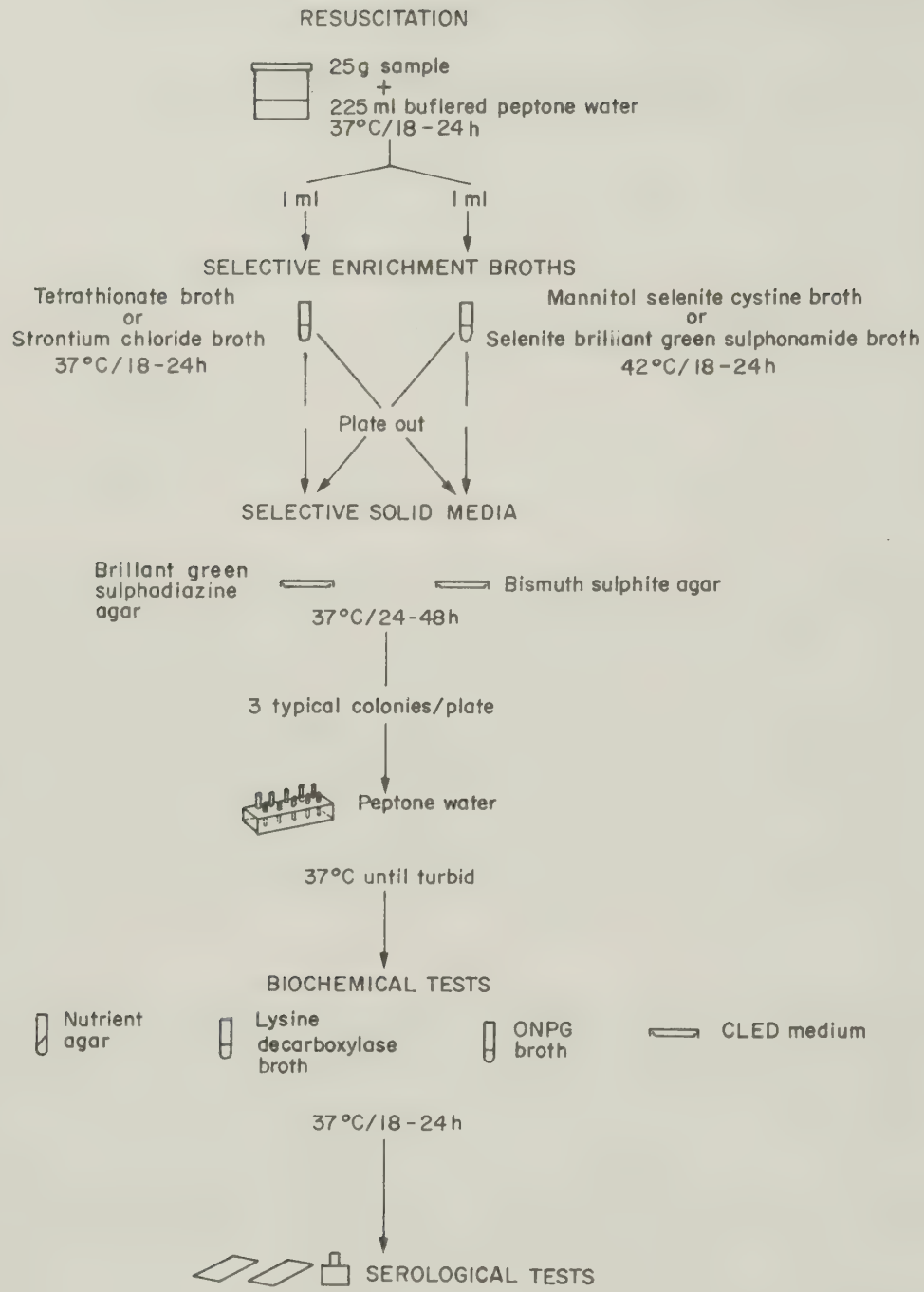

Figure 1 Flow diagram of examination procedures for salmonella

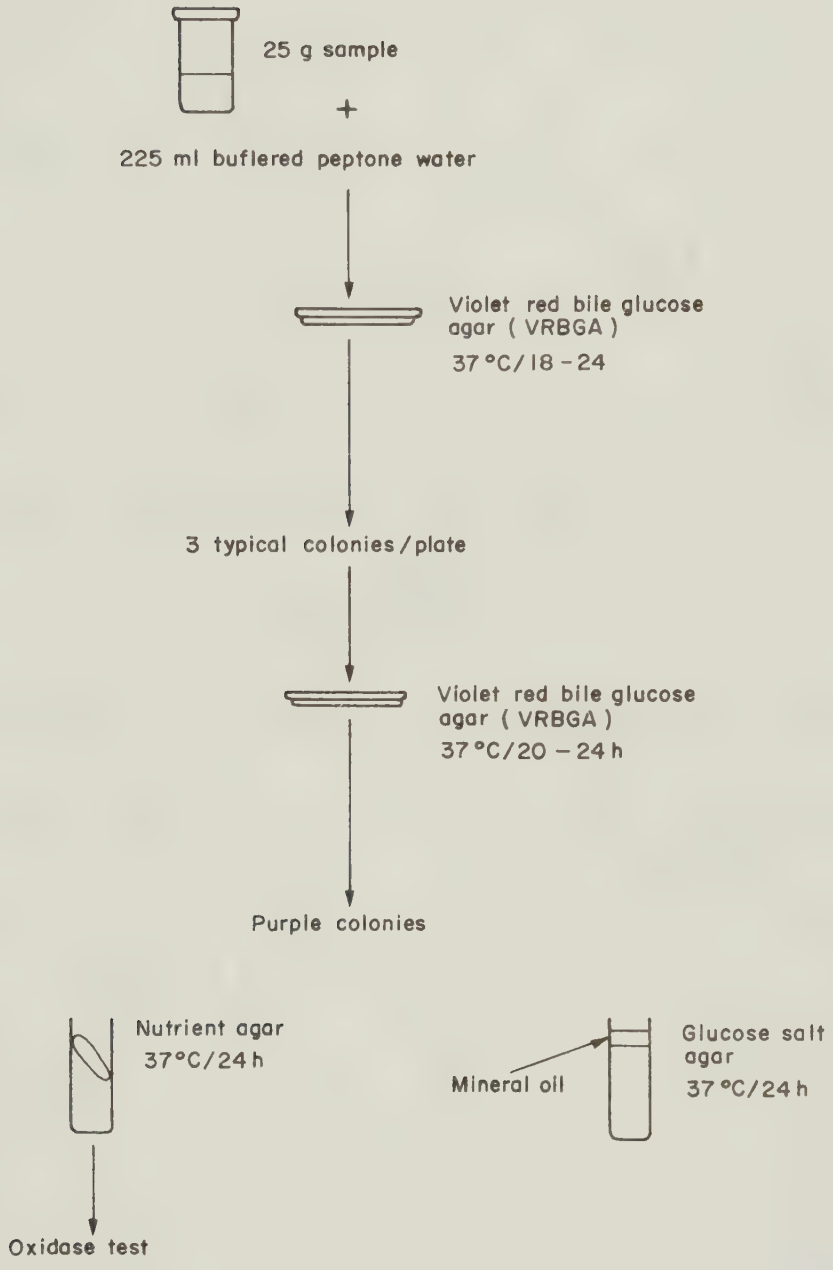

Figure 2 Flow diagram of examination procedures for enterobacteriaceae

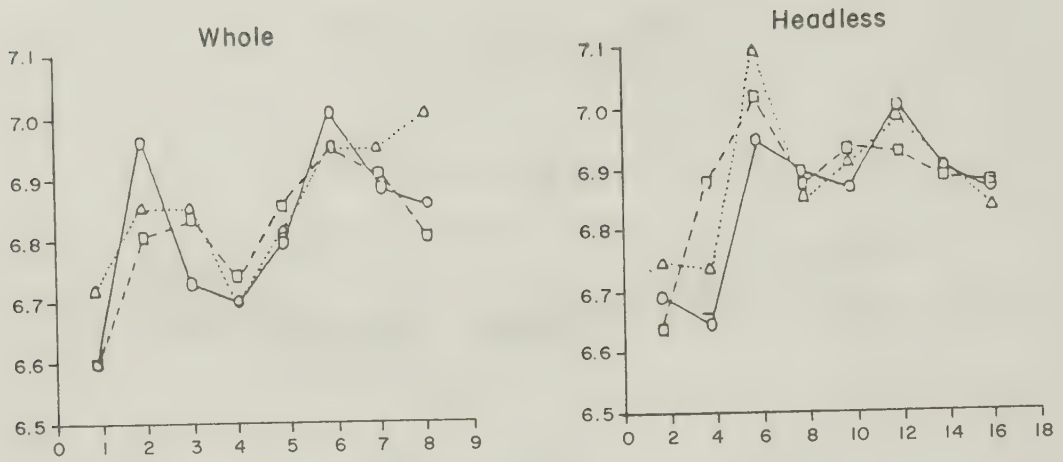

Figure 3 pH changes of P. monodon during iced storage

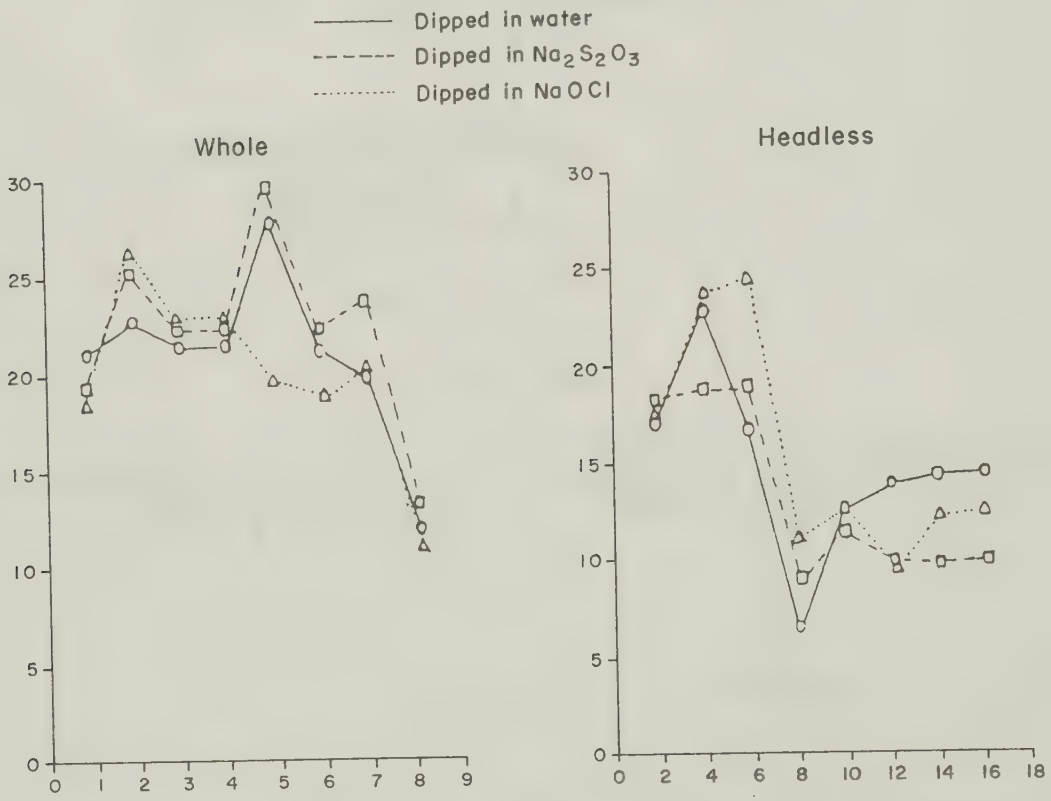

Figure 4 TVB changes of P. monodon stored in ice

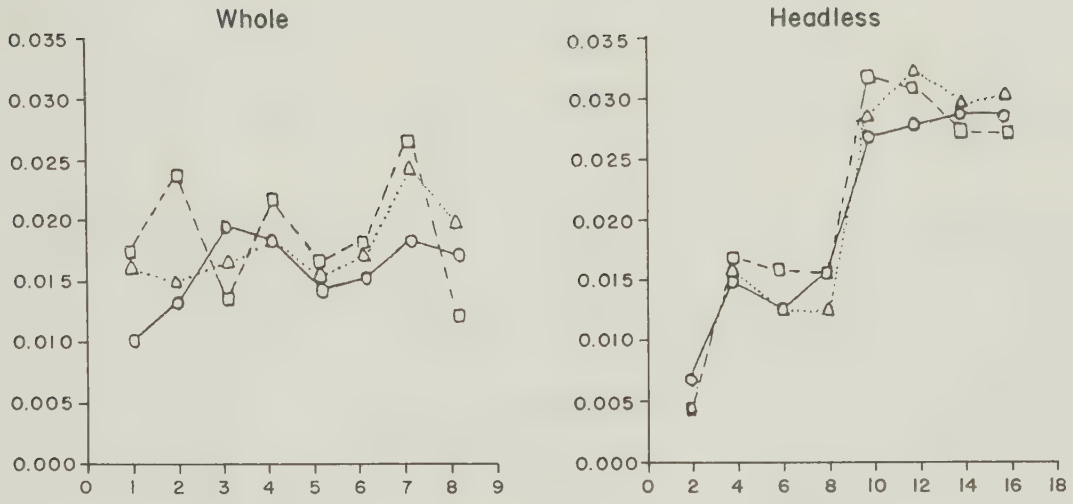

Figure 5 Ammonia changes of P. monodon during iced storage

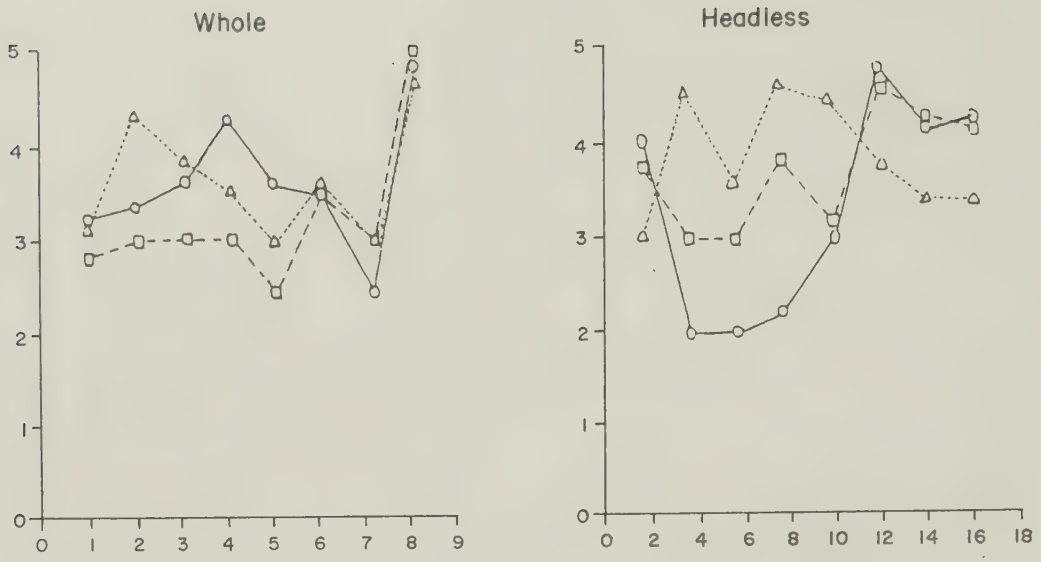

Figure 6 TPC changes of P. monodon stored in ice

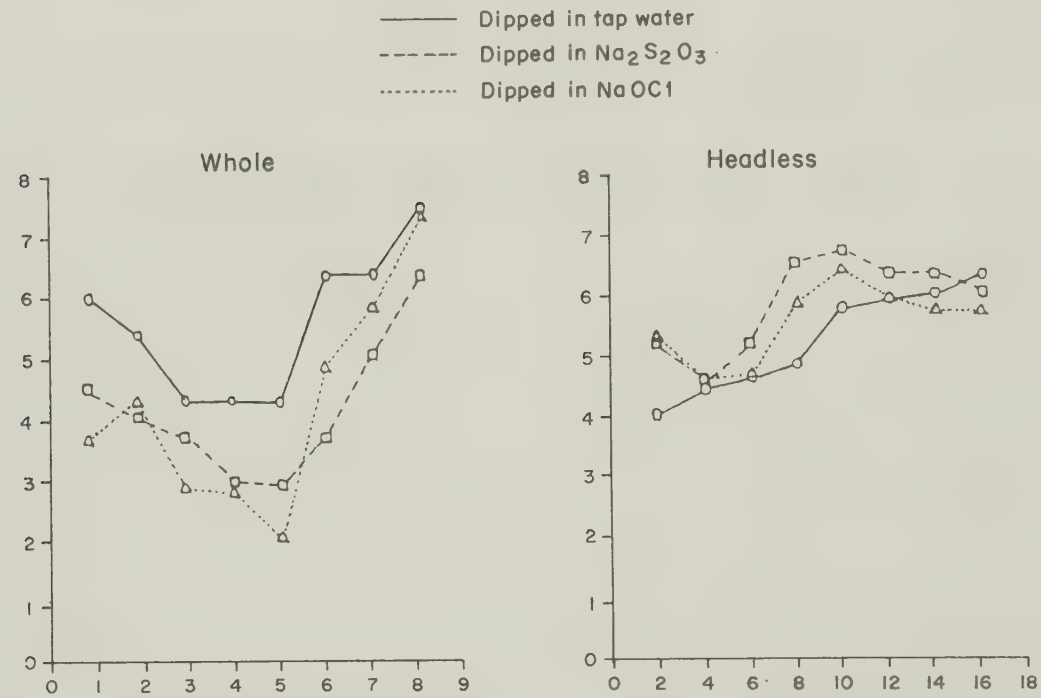

Figure 7 H_2S producers changes during iced storage of P. monodon

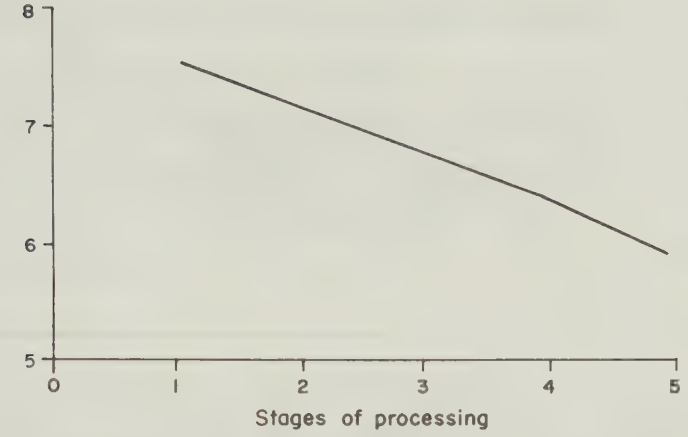

Figure 8 TBC changes during processing

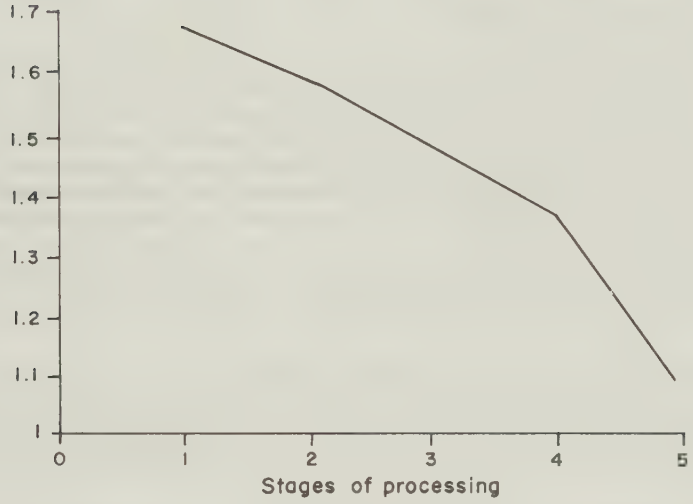

Figure 9 E.coli changes during processing

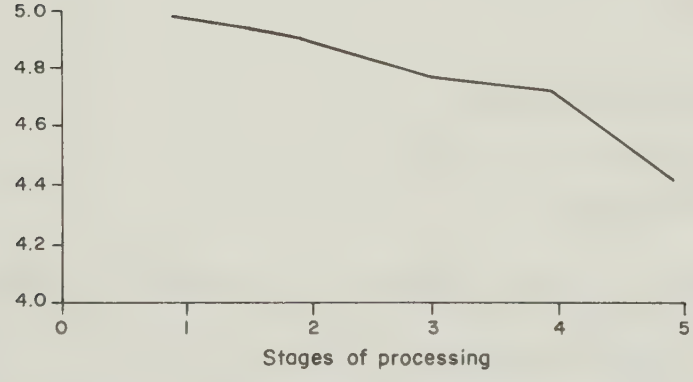

Figure 10 Staphylococci changes during processing

SALMONELLA CONTAMINATION IN THAI SHRIMP

by

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ABSTRACT

Salmonella contamination was investigated for farm raised shrimp (Penaeus merguiensis) and marine shrimp (P. merguiensis, P. monodon and Trachypenaeus) after harvest and at each step of processing and marketing. All samples were processed using pre-enrichment broth, then selective enrichment media before selective plating to isolate and purify suspect colonies. Biochemical screening was followed by serological typing.

Shrimp from local markets, landing places and seafood factories were also tested. Salmonella weltevreden and S. derby were found on fresh and frozen white shrimp and peeled and cooked marine shrimp. All the samples that were found to have salmonella were rechecked, but only the gut was collected for salmonella analysis. Of 31 good and medium quality shrimp samples tested for salmonella only two were found to have salmonella in addition to 17 white shrimp from local markets in Bangkok which originated from Samuth Sakorn landing port and also farm shrimp.

1. INTRODUCTION

The value of Thai shrimp export is more than 25 percent of the total value of fishery exports and the value of exports increased from $126.6 million in 1983 to $137.6 million in 1984. However, there still exists quality problems due to salmonella/shigella contamination, filth and decomposition. Some exports are rejected or discounted or detained by overseas import health authorities.

This study was initiated following reports of salmonella being associated with shrimp processed by GMP (Rattagool, 1986). The purpose of the study was to find technology and/or treatments to eliminate the salmonella/shigella contamination. Good manufacturing practice (GMP) is the most promising control method available to food processors.

2. MATERIALS AND METHODS

2.1 Sampling

A. Shrimp (P. merquiensis) from farm ponds

* Collected water from raised farm shrimp
* Shrimp after capture
* Shrimp after GMP was applied
* Frozen shrimp

B. Investigated Salmonella's location (sampling from local market, freezing plants and landing ports)

* Used outer shell matter for analysis
* Shrimp's faeces analysed

C. Peeling sheds

* Primary process - raw-peeled shrimp
  - cooked and peeled shrimp

* Factory peeling - peeled and cooked shrimp
  - shrimp after cooking

2.2 Microbiological Analysis

Standard Plate Count (SPC) were prepared and incubated at 20°C for 3-5 days and 37°C for 48 h. Enterobacteriaceae were determined by violet red bile glucose agar. Salmonella spp. were detected by 5 samples of 25 g each into 225 mL peptone buffer incubated 35°C 24 h then pool 10 mL from each peptone buffer into 450 ml cysteine and selenite broth for 37°c for 24, 48 and 72 h then streak one loop onto selective agar (brilliant green = bg, bismuth sulfite = bs, xylose lysine desoxycholate = XLD). Isolated colonies were biochemically screened (triple sugar iron agar, lysine decarboxylase broth, urea) then suspected colonies were confirmed by serological typing, (serology test was undertaken at Salmonella Centre, Department of Medical Science). Standard plate count (SPC) followed Australian Standard Methods (SAA, 1976).

3. RESULTS AND DISCUSSION

Raw peeled shrimp and whole shrimp coming to the factory were collected for salmonella analysis. Salmonella spp. were found associated with raw peeled shrimp and factory peeled shrimp.

Salmonella may be located internally or externally on shrimp. The other probability, raw shrimp was peeled in the same factory area, it is possible to contaminate by baskets, by hand, tools etc, or either way it was contaminated from peeling sheds already.

After good manufacturing practice (GMP) was applied the total viable count (TVC) was reduced from 10^6 CFU/g to 10^4 and 10^2 CFU/g in the cooked shrimp, but salmonella were found all through the processing line.

White shrimp (P. merquiensis) was tested for salmonella contamination. Both the normal shrimp supply to Factory A and incoming shrimp after treatment involving GMP were examined. Salmonella weltevreden was found in incoming shrimp to the factory and also in the deheaded shrimp sample which was treated with GMP and had a low TVC (6.4×10^4 CFU/g). Many of the frozen shrimp from private companies were analysed with results reported in Table 4. Salmonella contamination on frozen shrimp is of great concern and so this study not only examined frozen shrimp from the freezing plant but also the contributing factors which may lead to contamination, for example, pond water, container, stools, ice, wash-water as well as shrimp from local markets, landing ports and peeling sheds in an effort to locate salmonella contamination. Further studies of the gut microflora of shrimp is required.

4. REFERENCES

SAA (Standards Association of Australia). Methods for the Microbiological Examination of Food. 1976 Part 2: Examination for Specific Organisms. Sydney, SAA.

Table 1

Microorganisms on peeled and cooked shrimp during storage

Storage (days)	Total Viable Count (CFU/g)		Gram Negative rod (CFU/g)
	20°C	37°C	
LP	$1.0x10^4$	$1.55x10^4$	<10
1 LP	$8.1x10^3$	$7.2x10^3$	$8.3x10^1$
3 LP	$7.5x10^3$	$2.1x10^3$	$4.0x10^1$
4 LP	$6.5x10^3$	$4.6x10^3$	$1.1x10^3$
6 LP	$1.75x10^4$	$6.9x10^3$	$1.2x10^1$
7 LP	$1.03x10^5$	$2.9x10^4$	$6.4x10^3$
8 LP	$<3.0x10^5$	$1.8x10^6$	$1.57x10^5$
9 LP	-	$8.0x10^5$	-
LR	$1.30x10^4$	$1.55x10^4$	<30
1 LR	$9.8x10^4$	$7.8x10^3$	$1.7x10^1$
3 LR	$1.55x10^4$	$1.10x10^4$	$6.0x10^1$
4 LR	$6.0x10^4$	$4.5x10^4$	$1.20x10^3$
6 LR	$1.77x10^8$	$4.77x10^7$	$3.4x10^6$
7 LR	$1.16x10^9$	$7.0x10^8$	$>3.0x10^7$
8 LR	Reject	Reject	Reject

LP (factory packing)
LR (repacked as the retail market)

Table 2

Total viable bacterial count, Enterobacteriaceae and Salmonella of fresh peeled uncooked and peeled cooked frozen shrimp

Sample	TVC/g		Entero-bacteriaceae (CFU/g)	Salmonella
	GM	90% CI		
Incoming peeled and uncooked	$1.9x10^6$	$1.5x10^6$-$2.4x10^6$	$>3.0x10^4$	+
Frozen packed shrimp	$1.6x10^5$	$3.1x10^6$-$8.0x10^5$	$<3.0x10^2$	-

Salmonella derby was found

Table 3

Salmonella and Vibrio sp. isolation from incoming, deheaded and frozen shrimp

Sample	Salmonella sp.	Vibrio cholera	Vibrio parahaemolyticus
Incoming shrimp	-ve	-ve	4/5
Deheaded (on processing line)	-ve	-ve	3/5
Headless and shell on (frozen)	-ND	-ve	3/5

ND = not determined

Table 4. Microorganisms contamination on shrimp processing plant G,D (plant survey)(plant III and IV)

	TVC/g				*E. coli*		Enterobacteriaceae		Vibrio			
	20°C		35°C		cell/g		cell/g					
	GM	95% CI	GM	95% CI	GM	95% CI	GM	95% CI	cholera	para	algine	Salmo-ella
Shrimp in auction process at FMO	9.82×10^{6}	2.0×10^{6} - 1.97×10^{7}	7.26×10^{5}	2.0×10^{5} - 1.17×10^{6}	5.74×10^{3}	1.7×10^{3} - 1.0×10^{4}	1.75×10^{4}	1.2×10^{4} - 2.0×10^{4}	-ve	4/5	5/5	-ve
Shrimp kept in ice (plant C)	1.36×10^{6}	2.2×10^{6} - 7.0×10^{6}	1.39×10^{6}	1.2×10^{6} - 1.53×10^{6}	3.21×10^{3}	0.6×10^{2} - 3.4×10^{3}	1.83×10^{4}	1.4×10^{4} - 2.84×10^{4}	-ve	4/5	5/5	-ve
After washing and deheading (C)	1.57×10^{5}	9.2×10^{5} - 1.5×10^{6}	9.6×10^{5}	5.1×10^{5} - 2.4×10^{6}	2.62×10^{1}	1.0×10^{1} - 1.2×10^{2}	3.76×10^{4}	2.76×10^{4} - 6.2×10^{4}	-ve	5/5	5/5	-ve
Frozen shrimp (C)	4.13×10^{5}	2.4×10^{5} - 7.5×10^{5}	1.4×10^{5}	1.4×10^{5} - 4.7×10^{5}	0	0	4.06×10^{1}	1.5×10^{1} - 7.0×10^{1}	-ve	1/5	5/5	-ve
Shrimp kept in ice (plant D)	3.62×10^{5}	2.1×10^{5} - 5.0×10^{5}	2.4×10^{5}	1.29×10^{5} - 4.2×10^{5}	10	-	6.5×10^{1}	2.5×10^{1} - 1.15×10^{2}	-ve	5/5	5/5	-ve
Washed in water after deheading (D)	2.6×10^{5}	-	1.66×10^{5}	-	6.0×10^{1}	-	1.94×10^{3}	-	-ve	-	-	-ve
Ice used for shrimp	2.0×10^{7}	-	-	-	5.6×10^{1}	-	5.2×10^{4}	-	-ve	-	-	-ve
Wash water after sorting	6.10×10^{5}	-	3.0×10^{5}	-	1.55×10^{1}	-	2.0×10^{3}	-	-ve	-	-	-ve

Table 5

Salmonella sp. identified from Thai fishery products

S. weltevreden
S. anatum
S. blockley
S. typhimurium b
S. typhimurium
S. agona
S. derby
S. lexington
S. krefeld
S. newport
S. montevideo
S. emek
S. seftenberg
S. albany
S. london
S. rissen

Source: from WHO/National Salmonella Centre.
January-December 1987

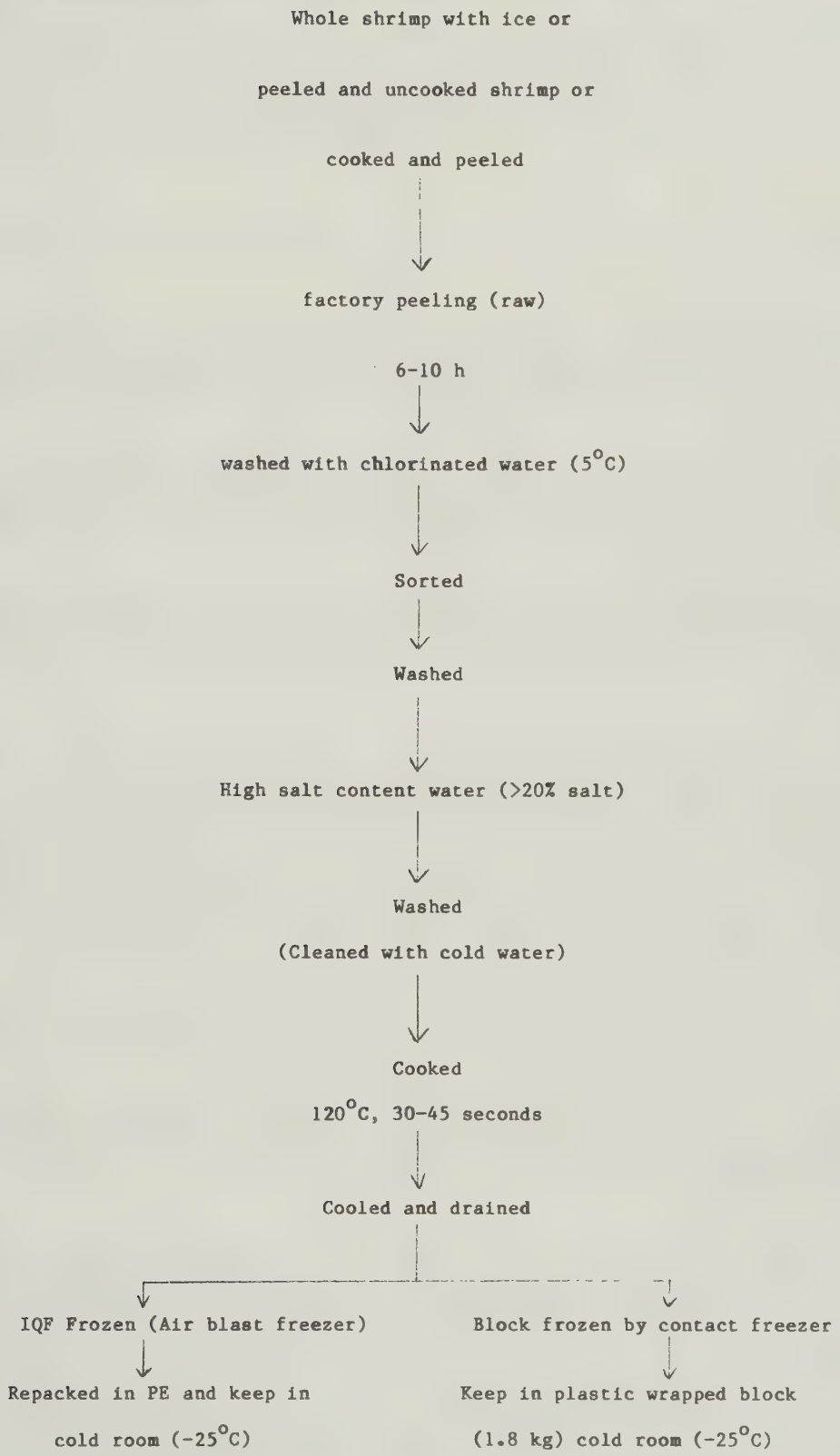

Figure 1. Process for peeled and cooked shrimp

MICROBIAL FLORA OF POND CULTURED PRAWN (PENAEUS MONODON)

by

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ABSTRACT

Prawn and water samples were collected from a prawn farm, on the west coast of Sri Lanka. Coliforms, Escherichia coli and Total Bacterial Count are reported for these samples. Vibrio parahaemolyticus, Salmonella and qualitative microflora are also reported for prawns.

The bacterial counts of prawns ranged from 2.0×10^4/g to 9.0×10^5/g whereas water ranged from 5×10^2/g to 8.8×10^3/g. E. coli ranged from 3 to 11/g for prawns and 3/100 mL to 49/100 mL for pond water. Salmonella was not detected. Only two samples were positive for V. parahaemolyticus. Bacterial counts showed no correlation with water salinity, which ranged from 14-18 ppt.

Gram positive bacteria were predominant in prawns and were represented by Micrococci (41.8%), Corynebacterium (19.3%) and Bacillus (14.2%). Common gram-negatives were Vibrio (19.2%) and Pseudomonas (5%).

1. INTRODUCTION

Up to a few years ago nearly all prawns harvested in Sri Lanka were derived from natural stocks in coastal areas. However, in certain east Asian countries shrimp culture is already a well established industry. Recently research into shrimp culture techniques has become a very important topic in Sri Lanka and the production of cultured shrimp has increased in the last few years. Along the west coast of Sri Lanka, low lying marshy areas are being converted into ponds, filled with brackish water and stocked with juvenile shrimp collected from hatcheries. The prawns are fed with a variety of foods, such as fish meal and rice bran, that are rich in protein. Ponds are harvested 3-4 months after stocking. Currently, most work on shrimp mariculture is focused on methods, which include improved pond design, to develop intensive culture system, feed formulation, hatchery production or juveniles and brood stock development.

Little or no information is available on microflora of pond cultured prawns in Sri Lanka. Such information is essential since microbial activity is one of the major causes of spoilage as well as a potential cause of food-borne illness and food poisoning.

This paper reports on (a) qualitative microbial flora of shrimp, (b) presence or absence of some important pathogenic and indicator organisms in cultured shrimp, and (c) dominant bacteria prevailing in shrimp collected from a series of ponds over a period of 10 months.

2. MATERIALS AND METHODS

Shrimp samples were collected from a shrimp culture farm located 40 miles north of Colombo in Thoduwawa. There were 34 ponds at the farm with total pond area of 10 hectares. Sampling for the study was done between January and September 1986. Shrimp samples were taken at approximately six week intervals. Five sub-samples, each of about 100 g, were taken from different areas of the ponds and then pooled to constitute one sample. Sample collection was routinely done from ponds about to be harvested.

The samples were collected into sterile polythene bags, sealed and then cooled in ice. Water samples were also collected into sterile bottles from the same ponds. All water and shrimp samples were transported in ice to the laboratory. Samples thus collected were analysed within four hours of sampling.

2.1 Microbiological Analysis

10 g samples of diced whole shrimp were accurately weighed aseptically into sterile stomacher bags and after addition of sterile peptone (90 mL) the contents were macerated for 30 s (shrimp extract). Serial dilutions of shrimp extract were spread on duplicate Nutrient Agar plates for Total Plate Count and incubated at 30±2°C/48 h. After incubation the plates were counted and twenty random colonies were picked and purified by restreaking on NA plates till pure colonies were obtained. These were then transferred onto NA slants and stored at 4°C for further characterisation.

Biochemical characterisation to generic level was carried out according to procedures and diagnostic schemes reported by Hobbs and Hodgkiss (1960), Lee and Pfeifer (1975) and Cowan and Steel (1977).

Total plate counts of water samples collected were carried out similarly by spreading aliquots (0.1 mL) from serial dilutions on duplicate NA plates and incubated at 30±2°C/48 h.

The most probable number (MPN) of total coliforms and Escherichia coli for shrimp and water samples were established according to Bacteriological Analytical Manual (BAM) (1978).

Salmonella isolation and identification was based on those tests listed by Andrews et al., (1978) and Collins and Lyne (1976). In the detection of Salmonella, Lactose Broth (LB) was used as pre-enrichment and Tetrathionate Broth and Selenite Cystine Broth were used in enrichment. For Salmonella isolation Xylose Lysine Desoxycholate Agar (XLD) was used). Further biochemical tests were used for identification.

Vibrio parahaemolyticus detection was carried out according to BAM (1978) and Collins and Lyne (1976). Glucose Salt Teepol Broth was used for enrichment, Thiosulphate Citrate Bile salts sucrose agar for isolation and Triple Sugar Iron Agar for screening. For identifications, biochemical tests were used and the MPN method was used in enumeration.

3. RESULTS

The results of the total bacterial counts, total coliforms, Escherichia coli, V. parahaemolyticus and Salmonella in the prawn samples are presented in Table 1. Table 2 gives the results of total bacterial counts, total coliforms and E. coli for pond water.

The total plate counts for prawn ranged from $0.2\text{-}9.1\times10^5$/g with a mean value of 3.2×10^5/g (Figure 1). The total plate count for pond water ranged from $0.5\text{-}8.8\times10^3$/g (Figure 2). A comparison of total bacterial counts for prawns and water is shown in Figure 3.

Organisms of public health significance such as total coliforms and E. coli were detected in very low numbers in prawns. Total coliforms were not detected in 25% of the samples, but when present, coliforms ranged from 7 to 75 coliforms/g. E. coli was not detected in 62.5% of the prawn samples, but when present, E. coli ranged from 4-11/g (Figure 4). The total coliforms for pond water ranged from 0.23-1.4/mL and E. coli ranged from .03-0.49/mL (Figure 4).

Vibrio parahaemolyticus was detected only in two samples (Figure 5). Salmonella was not found. Studies of the bacterial flora showed that gram positive organisms predominated. Over 75% of the flora consisted of gram positive organisms such as Micrococcus spp., coryneform bacteria and Bacillus spp. (Figure 6). The most common gram negative organism was Vibrio spp. While Pseudomonas and Moraxella were present in significant numbers and Enterobacterium, Flavobacterium and Aeromonas were also detected.

Conditions in the pond water were as follows: temperature from 28-32°C, pH between 7.4 and 8.2 and salinity from 14-18 ppt.

4. DISCUSSION

The mean bacterial counts of freshly caught pond-cultured prawns was 3.2×10^5 and were lower than the counts reported for marine prawns (3.9×10^6) from the Negombo Sea. The marine prawns were collected at landing sites 5-7 h after they were caught and immediately iced upon collection (Fonseka, 1985). The lower counts in the present study can be attributed mainly to the fact that immediate icing of cultured prawns after harvesting limits the likelihood of bacterial multiplication or subsequent contamination.

There is evidence to support the fact that surface water carries less bacteria compared to bottom mud about 50 ft below the surface (Williams et al., 1952). As prawns are bottom dwelling animals, the likelihood of their becoming contaminated with bacteria from the muddy substrate is always a possibility.

Deheading of prawns has lead to reduction of bacterial counts by 75% (Fiegar, 1950) and the effect of thorough washing on the reduction of microbial load of prawns has been documented by Green (1949) and Williams et al.,(1952). Good postharvest practices such as thorough washing with potable water, icing, deheading and good manufacturing practices would keep the total bacterial counts of processed pond reared prawns, to a reasonably low level.

The mean total bacterial counts of pond water was found to be below the mean bacterial counts for the prawns (Figure 3). The difference was found to be about 2 log values. This is probably an indication of the satisfactory quality of pond water.

Micrococcus, coryneform bacteria and Bacillus species predominated in pond reared prawns in this study. The marine prawns from Negombo also carried large numbers of Micrococcus but no coryneforms were detected there (Fonseka, 1985).

The high incidence of coryneform bacteria in prawns and shellfish from tropical (Sreenivasan, 1959) and temperate waters (Cann, 1977; Walker et al., 1970) has been reported. The work carried out on pond reared prawns (Penaeus stylirestris, Penaeus varnamei, Penaeus setiforus and Penaeus aztecus) in Texas by Vanderzant et al., (1971) and Christopher et al., (1978) further indicated the ubiquitous and common presence of coryneform bacteria. It may be possible that coryneform bacteria are closely associated with crustacean shellfish.

Two samples collected during this study were found to carry V. parahaemolyticus which is a halophilic, thermophilic and pathogenic organism (Qadri and Zuberi, 1977). It is essentially a coastal and estuarine organism and has only rarely been isolated from fish caught in the open sea (Barrow, 1976). It has been isolated from marine sediments, sea water, shellfish and finfish (Horie et al., 1967; Aoki et al., 1967). It is reported to be highly sensitive to processing temperatures and does not survive normal food processing (Asakawa, 1967).

Its growth is not only temperature dependent but is also influenced by the presence of organic nutrients such as that present in sewage (Liston, 1973). Thus, although its incidence in water in the coastal zone is usually seasonal, in estuarine waters rich in organic nutrient it is possible to detect it throughout the year (Barrow, 1976). While ingestion of a small number of this organism is apparently harmless, in as little as two hours they can multiply, resulting in a population capable of causing food poisoning (Liston, 1973), and is therefore considered a food poisoning agent. Food poisoning due to V. parahaemolyticus has been reported from many countries (Mitwani & Takela, 1976; Aoki et al., 1967; Qadri & Zuberi, 1977).

The low counts of V. parahaemolyticus detected in two samples of prawns in this study, would not constitute a major threat to the quality of pond reared prawns as subsequent washing, processing, freezing, cold storage or cooking within a short period of harvesting which will ensure elimination of the organism and thereby prevent any change of food poisoning.

Reilly et al.. (1984) working on brackish water prawns reported that V. parahaemolyticus was present commonly as part of the natural flora, but not isolated after processing.

The presence of Vibrios in pond reared shrimps (Christopher et al., 1978; Vanderzant et al., 1971) and fish and shellfish from estuarine and marine waters is well documented (Liston, 1957; Simidu et al., 1969; Surrendran and Gopakumar, 1982; Durairaj et al., 1983; Reilly, 1984). Vibrios were the next prominent group of gram negative bacteria detected in this study.

No clear relationship could be established between the variation of bacterial load of prawns and pond water characteristics such as salinity, temperature and pH. Some pond characteristics such as oxygen concentration and salinity can be controlled by management practices. The environmental conditions, feed type, rate of feeding, fertilisations of pond and aeration, probably would have contributed to slight variation in type and levels of microflora over the period of the study.

Coliform organisms, faecal coliforms and E. coli are primarily used to indicate some degree of potentially hazardous contamination based on the assumption that the natural habitat of the family (Enterobacteriaceae) to which these bacteria belong, is the faeces of man and other mammals, thereby indicating faecal contamination. E. coli is considered the most positive indicator of human faecal contamination. Presence of E. coli in a food implies the possibility of finding one or more of a wide diversity of enteric pathogens which may also have gained access to the food and hence introduce human health hazards. There is evidence to show that lagoon and inshore waters carry E. coli regularly. Cann (1977) reported that freshly caught penaeid shrimps do not carry E. coli but smaller inshore shrimp of parapeneopsis spp. collected at sea were contaminated with E. coli.

In this study 50% of the prawn samples had coliforms at less than 20 (MPN)/g while the rest of the samples carried coliforms below 75 (MPN)/g. E. coli was not detected in 82.5% of the samples. Even the E. coli positive samples had low counts. Counts of coliforms and E. coli in pond water were also found to be less than those in prawns. There are no microbiological standards for water

used in prawn culture in Sri Lanka, however, the coliforms standard for approved shellfish waters, described in the Manual of Operations of the USA National Shellfish Sanitation program, indicate a necessity for maintaining values so that: "The coliform median MPN of water does not exceed 70/100 mL and not more than 10% of the samples ordinarily exceeds an MPN of 230/100 mL". The coliform median MPN in this study is 79/100 mL and none of the samples had the coliform MPN over 140/100 mL which is well below 230/100 mL.

It should be pointed out here that though the coliform median MPN of pond water observed in this study was slightly above 70/100 mL, the particular American standard applies to molluscan shellfish, which feed by pumping large quantities of water using a filter mechanism. Thus this feeding process concentrates bacteria and plankton in the digestive trace of the animal. As such, exceeding the microbiological limits of 70/100 mL for coliforms by a small value in pond water samples should not be considered adverse in prawn culture.

It is evident from these findings that the cultured prawns and pond water do not carry very high total bacterial counts. Coliform counts and E. coli in these samples were not very high, while V. parahaemolyticus which is considered to be one of the important food pathogens in seafood, is not found in sufficiently large numbers to cause any problems. Salmonella was not detected in any of the samples. Under proper management and harvesting practices pond reared prawns can be used to produce a good quality wholesome product as good as the conventionally caught marine product.

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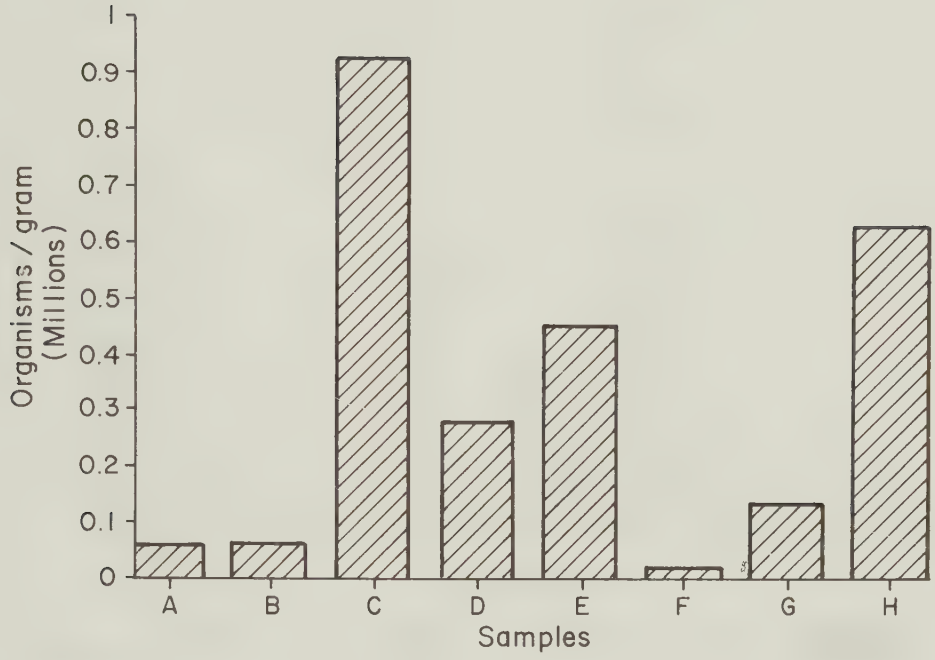

Figure 1 Microbiological data on cultured prawn -TBC

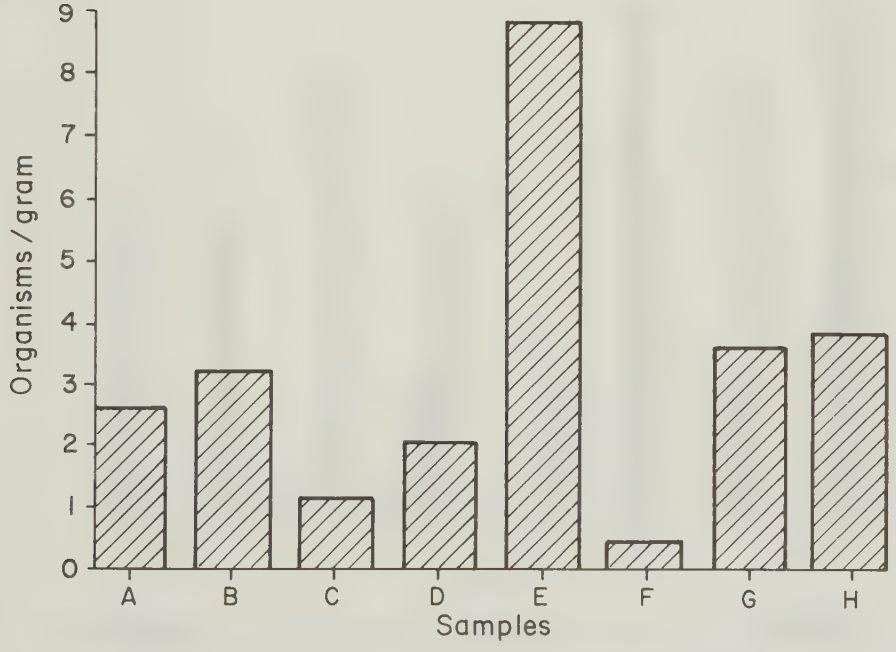

Figure 2 Microbiological data of pond water -TBC

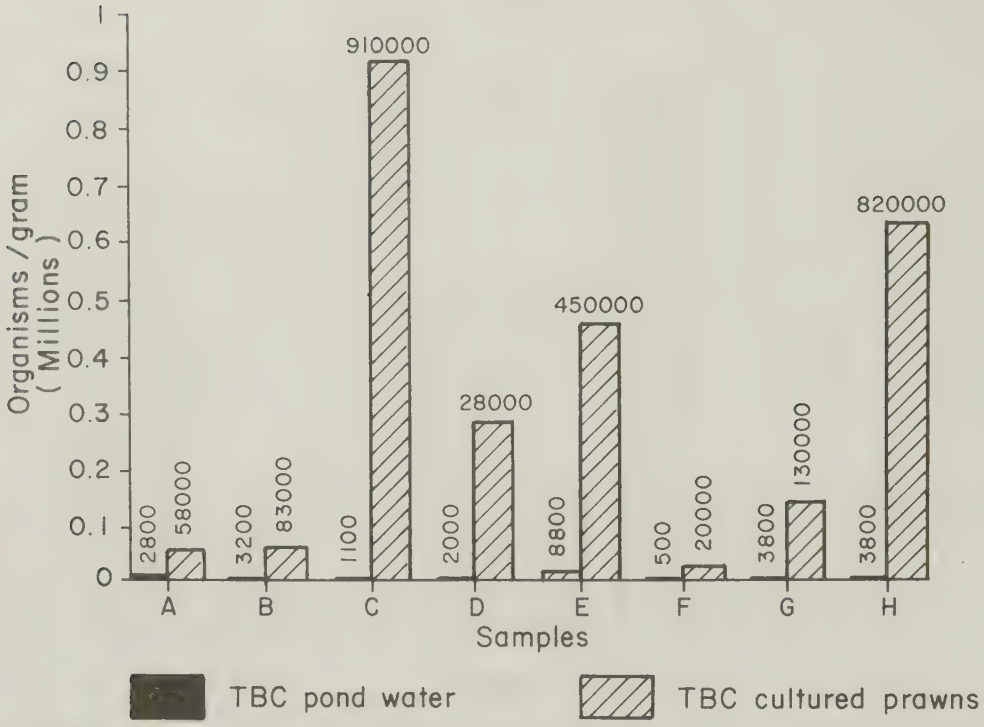

Figure 3 Comparison of TBC of prawns and water

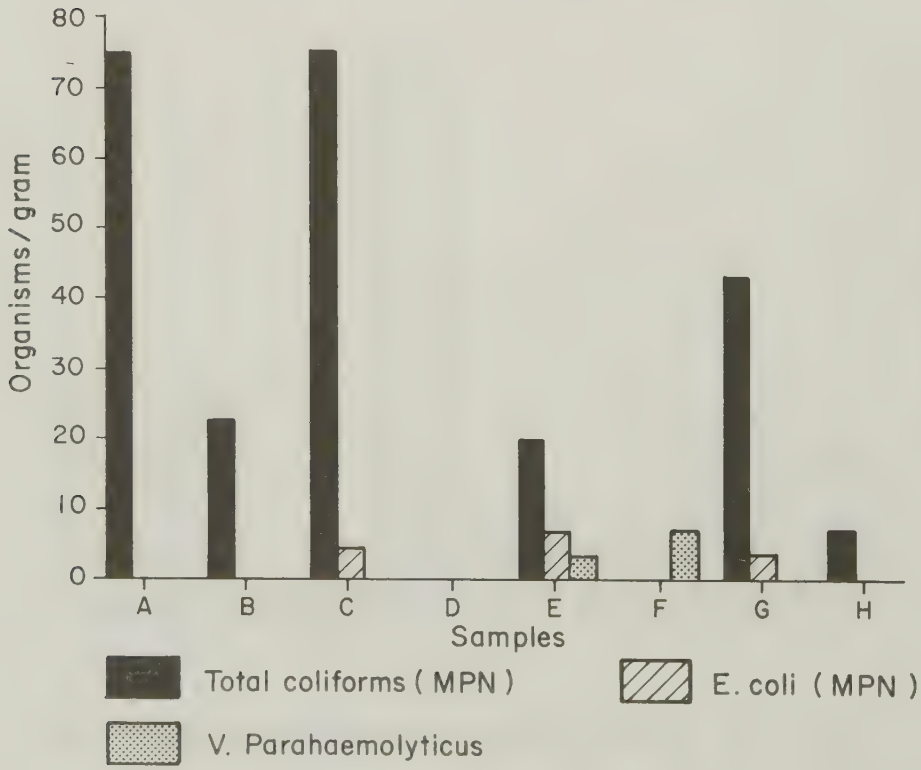

Figure 4 Microbiological data on cultured prawn

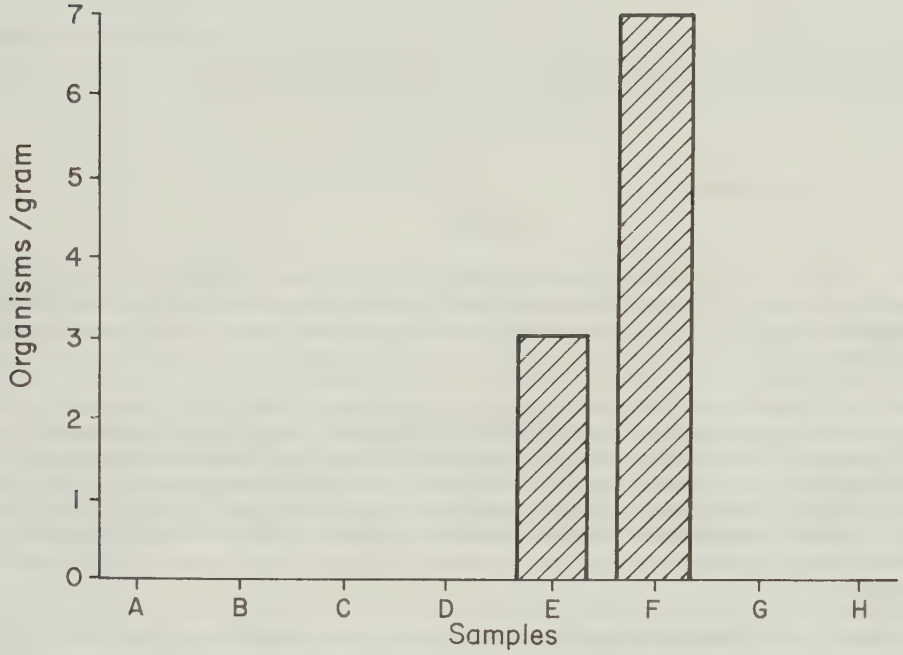

Figure 5 Vibrio parahaemolyticus in prawns

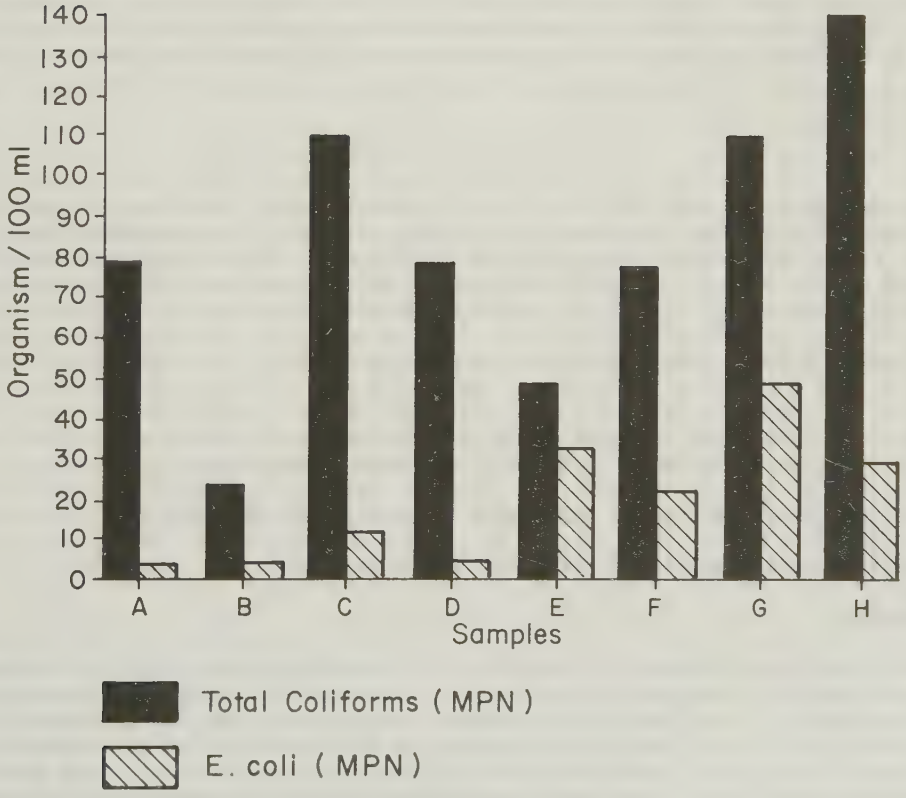

Figure 6 Microbiological data of pond water

HYGIENIC PROCESSING OF SHRIMP IN THAILAND

by

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ABSTRACT

In 1986, 28,729 t of shrimp worth $4,391 million was exported to USA, Japan, Australia and Europe. Some shrimp shipments were detained and rejected because of decomposition, filth, hazardous microorganisms and other sub-standard characteristics.

These studies used white shrimp (Penaeus merquiensis) and tiger shrimp (Penaeus monodon) from prawn raising farms at Samut Songkarm (80-100 km from Bangkok) and marine shrimp. Both species of shrimp were either treated or untreated (representing factory processing) by following the usual practices at the factory freezing plant. The final product was headless shell-on 1 kg uniform frozen blocks; treated shrimp were washed with cold clean (5°C) water (3:1 ratio of water and shrimp) then washed with chlorinated (30-50 ppm) water with 10 percent salt content.

Five treated and untreated samples were taken after the shrimp arrived at the factory and on the processing line (before and after washing, deheading, sorting, and before and after freezing). All the samples were subjected to chemical and microbiological analyses together with organoleptic evaluation using hedonic score sheet (appearance, colour, texture, taste, flavour). It was found that shrimp dipped for 1 min in 50 ppm chlorinated water had a total bacteria count of between 2-3 log cycles; it was also found that 40 ppm chlorine water was effective and gave a large reduction in E. coli and Enterobacteriaceae counts. K-value, total volatile nitrogen and pH were significantly different between the treated and untreated samples by good manufacturing practice (GMP) after holding for 15 h. The sensory evaluation results supported the analytical results.

INTRODUCTION

Frozen shrimp and other types of the fishery products, were detained or rejected by health authorities of various importing countries with problems such as: decomposition, high total viable bacterial count, filth, unexpected foreign matter and food-borne microbial pathogens. This study used appropriate techniques to control the quality of the raw material, for example shorter collection times (3 h instead of 5-6 h) and reducing the temperature as fast as possible with ice.

The objectives of this study into the hygienic processing of shrimp in Thailand were as follows:

1. To identify the factors that influence the hygienic condition of shrimp including microbial contamination and the cause of shrimp decomposition.

2. To find the proper processing techniques to improve the hygienic quality of shrimp.

3. To determine good handling and manufacturing standards.

MATERIALS AND METHODS

Samples of shrimp were collected at five in-line processing plants located in Bangkok, Samuth Prakarn and Samuth Sakorn. Farm shrimp from Samuth Sakorn (40 km from Bangkok) were collected then divided into 2 treatments. The laboratory treatment was washed after landing with ice water, then kept in adequate ice, the second portion was treated by GMP, where shrimp were washed with 2% brine solution (-2°C) twice for 3-5 min then kept in ice. Samples collected were: farm water, shrimp after capture, shrimp after icing, wash water and shrimp after GMP was applied. The factory samples were kept in inadequate ice (as is the normal commercial practice). At 6.00am the factory sample went to the auction place following normal commerical practices. Both the laboratory sample (L) and factory sample (F) were treated the same using one of two techniques, GMP (good wash, normal) or

poor or inadequate ice and water and F1 and L1 were processed by normal plant method (limited ice, one water wash) and F2 and L2 treated with GMP (brine solution, adequate ice and 5 times wash with water).

After washing, then all the treatments passed to the processing line. The GMP sample was processed under good conditions; washed with clean, cold water in the shortest possible time. Also proper freezing for this type of product (contact freezer). Every step of the in-line process was sampled (deheaded, sorted, weighed, soaked in 50 ppm Cl2 - water then replaced with 5 ppm Cl2 - water before freezing) for F1L1 and F2L2 and brought back to the laboratory.

All samples were analysed chemically, microbiologically and physically.

Chemical and Microbiological Analysis

Shrimp quality was measured by the chemical changes such as: total volatile base nitrogen (TVB-N), trimethylamine (TMA), pH and K-value using standard AOAC methods (1984). The microbiological quality was analysed using FDA methods (FDA, 1978). Total aerobic viable count (TVC), Enterobacteriaceae, coliforms and faecal coliform numbers were assessed. The food-borne pathogens, Salmonella sp., Vibrio sp. and Staphylococcus aureus were examined for their presence or absence. Salmonella was isolated and identified using 5 replicates of 25 g of sample into 225 g of pre-enrichment broth for 24 h at 37°C. Then 10 mL from each of five bottles was transferred into both 450 mL of selenite cystine broth (selective enrichment broth), and tetrathionate broth and incubated at 37°C for 24, 48 and 72 h, followed by selective enrichment medium to purify microorganism. Typical colonies were examined further by biochemical screening using triple sugar iron, lysine decarboxylase and urease agar before the suspect colonies were confirmed by serotyping.

Staphylococcus aureus was isolated directly using Baird-Parker tellurite egg yolk agar, incubated at 37°C for 48 h. Typical colonies were confirmed by rabbit plasma for coagulase production.

The thioglycolate citrate bile glucose salt agar (TCBS) was used to detect Vibrio sp. The typical colonies were re-confirmed using salt concentration as well as biochemical analysis, then suspect colonies were checked by serotyping.

RESULTS AND DISCUSSION

It was found that the total viable count (TVC) of shrimp farm water was 1.4×10^6 CFU/mL and E. coli was 1.5×10^2 CFU/mL, while brine (10% salt content) solution had a TVC of 2.0×10^4 CFU/mL and E. coli was zero, while tap water had a TVC of 5.6×10^2 and E. coli of zero (Table 4).

Pond raised white shrimp (P. merquiensis) and tiger shrimp (P. monodon) after harvest (farm and marine shrimp) and after landing showed a high level of bacterial contamination (Table 5). It was found that practical plant techniques to reduce microbial contamination included the use of cold, clean water. 10% salt solution with adequate ice (-2°C) or 30-50 ppm chlorinated water (2-5°C) and a proportion of water to shrimp of 3 to 5:1. Table 5A shows that the TVC of all farm and marine shrimp had similar levels of microbial contamination. Results showed contamination of 5.6×10^6 CFU/g when shrimp arrived at the factory and reduced to 5.9×10^4 CFU/g, 4.7×10^4 CFU/g and 2.1×10^4 CFU/g respectively for the sample after washing in clean water, then with 10% salt and finally with 50 ppm Cl2. Results for white shrimp (farm and marine) or tiger shrimp (marine) showed that the total viable bacterial number declined 2-3 log cycles between when shrimp arrived at the factory through the final wash and freezing of shrimp. Enterobacteriaceae count reduced 1-2 log cycles as shown in Table 5B and E. coli showed similar reductions (Table 5C). Table 6A and 6B show the results for TVC covering the processing of shrimp after washing in cold clean water, dehead and sorted (10 min dip into 50 ppm chlorinated water) before freezing and frozen product are 3.76×10^6 CFU/g, 1.0×10^5 CFU/g, 2.0×10^4 CFU/g and 1.76×10^4 CFU/g respectively. Enterobacteriaceae also demonstrated a large reduction in numbers (4 log cycles). These results demonstrate that a wash with clean water and proper handling techniques will reduce high TVC, Enterobacteriaceae, coliform and E. coli counts. Tables 7, 8 and 9 show result of chemical indices (TVB, indole, pH and K-value) for shrimp left at room temperature (28±5°C) in a basket for 27 h. Between 9-18 h storage, a big change on indole, TVC, K-value and pH occurs, which corresponds with the growth of microorganisms during the lag phase. Table 11 and 12 demonstrate that low temperature (-18°C) together with a good wash, especially under GMP, can maintain shrimp quality. K-value increased from 11.18% to 15.87% after 5 months frozen storage, TVC increased from 3.97 to 4.10 after 5 months and samples B, C, and D have initial TVC values of 3.54, 4.02 and 4.55 respectively which increased to 4.40, 4.46 and 6.01. The results show that good quality shrimp given a poor quality wash will suffer the same 2 log increase in microbial load after 5 months storage as average quality shrimp after the same poor quality wash. Conversely average quality shrimp given a good wash demonstrated a minimal increase in microbial load.

The composition of the flora on marine and farm shrimp have been indentified from plate count agar after 5 days at 20°C (Table 10) and shows acinetobacter (12%), flavobacterium (13%), moraxella (10%) and few Pseudomonas (1%) on marine shrimp, compared to the organisms which were found on farm shrimp; corynebacterium (33%). More spoilage bacteria were found on marine shrimp than on farm shrimp. Both shrimp have been given the GMP application but the better technique gave a longer shelf life, 10 days instead of 7 days using ice preservation.

CONCLUSIONS

This study demonstrated the benefits of reduced handling delays, proper icing, good hygienic practices, proper plant operation techniques in maintaining the quality of frozen shrimp.

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Table 1

Shrimp exports from Thailand (1986)

Country (tonne)	Frozen shrimp (10^6 baht)	Cooked shrimp (10^6 baht)	Dried shrimp
Hong Kong	-	72.1	72.1
Japan	2092	31.0	31.0
USA	1209	26.2	26.2
Malaysia	-	-	-
Australia	-	86.2	-
UK	-	-	-
The Netherlands	-	60.0	-
Others	-	-	-

Table 2

K-value, TVB of fresh and frozen white shrimp (*P. merquiensis*) (farm shrimp) in normal situation (plant A, B, C, D)

Species (%)	pH (mgN/100 g sample)	K-value	TVB
Fresh	6.84 (6.44-7.24)	23.72	11.79 (10.76-13.23)
Medium (quality)	6.98 (6.60-7.30)	26.03	16.08 (12.26-18.82)
Frozen	7.19 (7.09-7.36)	32-05	9.82 (9.04-10.6)

Table 3. pH, Total Volatile Nitrogen, K-Value and Indole of tiger and white shrimp from freezing plant (A) normal situation from 6 replications

Codes	pH	TVB mgN/100 g sample	K-Value %	Indole µg/100g	TVC/g 20°C	TVC/g 37°C
White shrimp (farm)	6.86	12.46	21.79	1.3	x10	x10
White shrimp (marine)	6.98	25.24	34.45	3.4	x10	x10
Tiger shrimp (marine)	6.96	11.37	20.20	0.6	x10	x10

Table 4. Microorganisms contaminating the water in shrimp farms, brine solution (10 percent) and tap water (1 MK-farm)

Sample	TVC/mL		*E. coli* (CFU/mL)	Entero-bacteri-aceae (CFU/mL)	MPN	
	20°C	37°C			Coli-form	*E.coli*
Water from farm pond	1.44×10^6	6.08×10^6	1.5×10^3	8.01×10^4	180	25
Brine solution	2.0×10^4	2.33×10^3	0	2.56×10^3	35	17
Tab water for washing and cleaning after brine	5.6×10^2	4.16×10^3	0	7.5×10^0	0	0

Table 5A. Microorganisms contaminating shrimp (white shrimp) before processing and on the processing line

	TVC (CFU/g)											
	Farm shrimp				Marine shrimp							
	White shrimp				White shrimp				Tiger shrimp			
	20°C		37°C		20°C		37°C		20°C		37°C	
Types	GM	95% CI	GM	95% CI	GM	95% CI	GM	95% CI	GM	95% CI	GM	95% CI
Arrived at factory	5.6×10^6	1.53×10^6 – 1.26×10^7	2.33×10^6	7.2×10^5 – 7.08×10^6	2.05×10^5	1.99×10^5 – 5.88×10^5	1.03×10^6	6.1×10^5 – 1.77×10^6	7.51×10^6	4.5×10^5 – 1.3×10^6	5.5×10^5	3.3×10^5 – 9.8×10^5
Afer washed and cleaned	5.8×10^4	2.5×10^4 – 9.3×10^4	4.48×10^4	3.9×10^4 – 4.9×10^4	1.03×10^5	2.14×10^4 – 4.04×10^5	3.76×10^5	1.76×10^5 – 5.42×10^5	7.0×10^4	3.9×10^4 – 1.35×10^5	4.38×10^4	3.55×10^4 – 1.07×10^5
10% salt washed	4.73×10^4	3.3×10^4 – 9.5×10^4	3.18×10^4	9.8×10^4 – 4.5×10^4	2.12×10^5	1.0×10^5 – 3.0×10^5	3.1×10^4	1.5×10^4 – 4.88×10^4	1.0×10^5	4.07×10^4 – 2.41×10^5	4.18×10^4	4.07×10^4 – 2.91×10^5
Cl_2 (50 ppm) washed	2.07×10^4	1.71×10^4 – 2.76×10^4	2.76×10^4	6.03×10^3 – 3.07×10^4	5.43×10^4	3.21×10^4 – 9.06×10^4	4.2×10^4	2.6×10^4 – 5.42×10^4	1.06×10^4	7.26×10^3 – 1.35×10^4	5.8×10^4	4.3×10^4 – 7.55×10^4

Table 5B. Enterobacteriaceae counts in farmed and marine shrimp before and during processing

	Enterobacteriaceae (CFU/g)					
	Farm shrimp		Marine shrimp			
	White shrimp		White shrimp		Tiger shrimp	
Sample	GM	95% CI	GM	95% CI	GM	95% CI
Incoming shrimp to factory processed	$3.19x10^5$	$1.20x10^5$-$6.4x10^5$	$2.13x10^4$	$8.4x10^3$-$5.82x10^4$	$4.4x10^5$	$1.2x10^5$-$3.1x10^4$
After wash with clean and cold water (0°C)	$1.94x10^5$	$6.1x10^4$ -$3.58x10^5$	$1.15x10^3$	$4.5x10^2$-$2.10x10^3$	$1.23x10^4$	$3.0x10^3$-$3.1x10^4$
Washed with 10% salt at -2°C	$2.04x10^4$	$1.66x10^4$-$2.3x10^4$	$2.71x10^4$	$1.0x10^3$-$9.85x10^4$	$9.92x10^3$	$6.6x10^3$-$1.1x10^4$
1 minute dipped in 50 ppm Cl_2 water at 1°C	$4.52x10^4$	$1.5x10^4$-$8.2x10^4$	$1.55x10^3$	$6.1x10^2$-$2.4x10^3$	$5.0x10^3$	$1.1x10^3$-$9.8x10^3$

Table 5C. *E. coli* count for farmed and marine shrimp before and during processing

Sample	*E. coli* (CFU/g)					
	Farm shrimp		Marine shrimp			
	White shrimp		White shrimp		Tiger shrimp	
	GM	95% CI	GM	95% CI	GM	95% CI
Shrimp at landing place arrived at plant	$2.58x10^2$	$1.2x10^2$-$4.5x10^2$	$3.6x10^2$	0 -$1.2x10^3$	$1.0x10^1$	0 -$2.0x10^1$
After normal wash proper wash	$2.0x10^1$	$1.0x10^1$-$5.5x10^1$	$4.28x10^3$	$2.0x10^2$-$9.6x10^3$	$1.0x10^1$	0 -$3.0x10^1$
Deheaded (L)	$2.6x10^1$	$4.0x10^1$-$1.0x10^1$	$3.46x10^2$	$1.3x10^2$-$5.4x10^2$	<10	<10
Deheaded (P)	-	-	$3.1x10^2$	$1.0x10^1$-$1.15x10^3$	-	-
Before freezing (L)	$1.03x10^2$	$3.0x10^1$-$2.0x10^2$	-	-	<10	<10
Before freezing (P)	-	-	-	-	-	-
Chlorine water washed (50 ppm)	$4.2x10^1$	$2.0x10^1$-$9.0x10^1$	$1.76x10^2$	$2.9x10^2$-$9.0x10^1$	<10	<10

Table 6A. Microorganism contaminating shrimp (white shrimp) before processing and on the processing line

Sample	Total Viable Count/g 37°C Geometric mean	37°C 95% Confidence interval	20°C Geometric mean	20°C 95% Confidence interval
Incoming whole shrimp	$3.76x10^6$	$2.41x10^6$-$7.94x10^6$	$6.42x10^6$	$1.44x10^6$-$9.12x10^6$
After washing (clean cold water)	$1.0x10^5$	$4.2x10^4$-$1.3x10^5$	$1.1x10^6$	$7.0x10^5$-$1.52x10^6$
Deheaded-sorted before freezing	$2.9x10^4$	$1.65x10^4$-$6.9x10^4$	$2.3x10^5$	$1.1x10^5$-$4.8x10^5$
Headless shell frozen shrimp	$1.76x10^4$	$9.3x10^3$-$2.5x10^4$	$2.96x10^4$	$1.3x10^4$-$7.3x10^4$

Table 6B. Microbial contamination of farmed white shrimp

	Enterobacteriaceae (CFU/g)		*Staphylococcus aureus* (CFU/g)	MPN Coliform		MPN *E. coli*	
	GM	95% CI	GM	GM	95% CI	GM	95% CI
Incoming whole shrimp	$4.7x10^5$	$1.7x10^4$-$6.8x10^5$	$2.2x10^2$	180	180-180	6.25	4.5-8.0
After washing	$6.2x10^3$	$8.0x10^2$-$2.08x10^4$	$4.0x10^1$	150	90-180	1.74	1.1-2.5
Deheaded sorted before freezing	$7.1x10^3$	$3.4x10^2$-$3.3x10^4$	$4.0x10^1$	3.96	1.3-8.0	0.5	0.2-0.8
Headless shell-on frozen shrimp	$1.6x10^1$	$1.0x10^1$-$3.0x10^1$	$4.0x10^1$	3.04	1.7-4.5	0.28	0.0-0.5

Table 7. The chemical-microbiological properties of tiger shrimp after being caught and kept frozen for 7 days and left at room temperature (20°±3°C) for 27 hours (1 NK-farm)

Storage time (hours)	pH	TVB mgN/100 g sample	Indole µg/100 g sample	K-Value %	TVC/g 20°C	TVC/g 37°C	Enterobacteriaceae CFU/g
1	6.55	7.80	2.0	6.64	4.40×10^4	2.99×10^4	7.4×10^2
3	6.67	12.01	2.0	11.64	2.15×10^4	1.15×10^4	1.98×10^3
6	6.74	14.41	-	14.44	7.05×10^4	3.5×10^4	1.81×10^3
9	7.10	12.91	-	21.06	1.32×10^5	4.17×10^4	3.02×10^3
12	7.32	23.41	-	25.39	2.86×10^6	4.67×10^5	1.81×10^5
15	7.29	27.31	-	44.06	4.84×10^7	4.37×10^7	2.40×10^7
18	7.02	32.72	24.40	48.23	7.21×10^7	6.97×10^7	3.10×10^7
21	7.12	42.02	-	49.32	3.01×10^8	1.18×10^8	4.98×10^7
24	7.36	75.62	93.71	52.75	1.61×10^8	1.78×10^8	1.57×10^8
27	7.44	91.85	98.86	63.86	3.68×10^8	3.85×10^8	2.56×10^8

Table 8. Temperature and pH of tiger farm shrimp levels when left at room temperature (1 MK-farm)

Hours after capture	Temperature (°C)	pH
0		
3	16	6.58
6	23	6.73
9	25	6.83
12	25	6.77
15	22	7.29
18	24	6.55
24	25	6.67
27	25	6.36
31	25	6.51

Table 9. Microbiological changes when frozen shrimp has been thawed then left 27 hours at room temperature (28°±3°C) (plant B-II)(1 MK-farm)

Time (hr)	TVC/g 20°C GM	TVC/g 20°C 95% CI m	TVC/g 20°C 95% CI M	TVC/g 37°C GM	TVC/g 37°C 95% CI m	TVC/g 37°C 95% CI M	*E. coli* CFU/g GM	*E. coli* CFU/g 95% CI m	*E. coli* CFU/g 95% CI M	Enterobacteriaceae CFU/g GM	Enterobacteriaceae CFU/g 95% CI m	Enterobacteriaceae CFU/g 95% CI M
0	4.40×10^{4}	3.01×10^{4}	5.39×10^{4}	2.99×10^{4}	2.19×10^{4}	3.9×10^{4}	1.12×10^{2}	8.0×10^{2}	1.5×10^{2}	7.4×10^{2}	4.35×10^{2}	1.65×10^{3}
3	2.15×10^{4}	1.15×10^{4}	4.29×10^{4}	1.51×10^{4}	9.55×10^{3}	3.29×10^{4}	1.7×10^{1}	5.0×10^{0}	3.5×10^{1}	1.98×10^{3}	1.85×10^{2}	9.0×10^{3}
6	7.05×10^{4}	3.08×10^{4}	1.23×10^{5}	3.5×10^{4}	1.5×10^{4}	8.05×10^{4}	2.1×10^{1}	1.0×10^{1}	3.5×10^{1}	1.81×10^{3}	6.0×10^{2}	4.7×10^{3}
9	1.32×10^{5}	6.65×10^{4}	2.67×10^{5}	4.17×10^{4}	2.65×10^{4}	5.4×10^{4}	1.92×10^{2}	1.0×10^{2}	2.95×10^{2}	3.02×10^{3}	1.45×10^{3}	4.45×10^{3}
12	2.31×10^{6}	7.2×10^{5}	3.09×10^{6}	4.67×10^{5}	2.34×10^{5}	1.05×10^{6}	3.33×10^{1}	3.0×10^{1}	4.0×10^{1}	1.81×10^{5}	8.8×10^{4}	3.88×10^{5}
15	4.84×10^{7}	3.74×10^{7}	8.0×10^{7}	4.37×10^{7}	2.84×10^{7}	8.0×10^{7}	5.51×10^{3}	3.7×10^{3}	7.25×10^{3}	2.4×10^{7}	1.0×10^{7}	4.0×10^{7}
18	7.21×10^{7}	1.74×10^{7}	1.2×10^{8}	6.98×10^{7}	1.52×10^{7}	1.2×10^{8}	1.61×10^{3}	2.9×10^{2}	2.5×10^{3}	3.1×10^{7}	2.0×10^{7}	4.0×10^{7}
21	3.0×10^{8}	1.9×10^{7}	2.97×10^{7}	1.18×10^{8}	0.128×10^{7}	1.51×10^{8}	6.59×10^{3}	2.0×10^{2}	6.68×10^{3}	4.98×10^{7}	7.1×10^{8}	2.0×10^{8}
24	1.61×10^{8}	1.32×10^{8}	1.83×10^{8}	1.78×10^{8}	1.62×10^{8}	1.94×10^{8}	2.66×10^{3}	3.25×10^{2}	4.8×10^{3}	1.57×10^{8}	1.2×10^{8}	2.0×10^{8}
27	3.63×10^{8}	1.61×10^{8}	5.59×10^{8}	2.85×10^{8}	1.56×10^{8}	4.2×10^{8}	4.24×10^{4}	1.7×10^{4}	7.2×10^{4}	2.56×10^{8}	1.08×10^{8}	4.36×10^{8}

GM = Geometric mean
m = minimum bacterial count
M = maximum bacterial count

Table 10. Microbes identified from marine and farm shrimp, after applying GMP from private processing line (1 MK-farm)

Microorganisms	% microbes from 20°C plate count	
	Marine [1]	Farm [2]
Micrococcus	40.00	40.90
Corynebacterium	19.00	32.94
Acinetobacter	12.00	-
Flavobacterium	13.00	2.27
Moraxella	10.00	5.68
Staphylococcus	2.00	3.40
Lactobacillus	1.00	-
Pseudomonas	1.00	-
Nanthomonas	-	-
Alcaligenes	-	-
Others	2.00	9.09
Total	100.00	88.00

1 = shrimp was preserved 7-10 days with ice
2 = shrimp was preserved 10-12 hours in ice before analysis

Table 11. Microbiological analysis of frozen white shrimp before and after shipment from Thailand to Sydney (Australia) by sea freight (plant B)

Type	TVC (CFU/g) as log 10			*S. aureus* coagulase CFU/g	Coli-form CFU/g	*E.coli* CFU/g
	before shipment	after shipment trial 1	after shipment trial 2			
A = good quality GMP technique	3.97	NA	4.10	<3	<2	<2
B = good quality but normal wash technique	3.54	NA	4.403	<3	<2	<2
C = Common quality found in market and in plants	4.02	NA	4.46	<3	<2	<2
D = poor quality normal wash technique	4.55	NA	6.01	<3	7	4

Note:

The trial shipment of frozen shrimp (white farm shrimp) was analysed at the Australian Atomic Energy Commission at Lucas Heights, Sydney, by Dr P.A. Wills and C. Banos, February 1986. It took 2 months for the ocean voyage; it was stated that the product quality met Australian microbiological standards (AMS) for imported frozen seafoods. The shipping trials to Sydney provide a clear picture about shrimp exports; all shrimp, even the lower quality, were able to meet the Australian Standards but the poor quality shrimp (D) is not recommended to be exported.

Salmonella was not found.

NA = not available yet.

Table 12. The K-value of frozen shrimp (farm) during frozen storage (-18°C) for 5 months, expressed in percentage (plant B)

Codes	1 day	2 months	3 months	4 months	5 months
A	11.18	9.83	14.37	13.15	15.87
B	18.38	23.25	21.34	24.53	28.81
C	25.63	27.21	25.75	25.43	-
D	40.00	45.14	44.80	48.39	-

A = good quality and GMP
B = good quality but normal wash
C = normal quality (medium) and good wash
D = poor quality and normal wash

IMPACT OF HANDLING PRACTICES ON THE MICROBIAL QUALITY OF SHRIMP (METAPENAEUS DOBSONI)

by

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ABSTRACT

Microbiological evaluation of tropical prawn Metapenaeus dobsoni was made at five key points during pre-process handling before freezing. The microbial count of fresh uniced prawn ranged from 4.8×10^5/g to 1.08×10^6/g. The initial microbial flora of fresh prawn in the order of predominance was Moraxella, Acinetobacter, Vibrio, Pseudomonas, Flavobacterium, Micrococcus and Bacillus species. The different processing practices influenced microbial flora. The microbial count fell after the beheading and washing steps, while it rose after peeling and deveining. Peeling and deveining caused an enormous increase in gram positive types, especially the Micrococcus species. The high increase in gram positive cocci reflects the extent of contamination due to human handling.

1. INTRODUCTION

The shellfish in their natural habitat may carry commensal bacterial flora, the composition of which is governed by factors such as feed, habitat or season. Apart from the bacteria naturally associated with the shrimp, considerable numbers get access to flesh as contaminants during different stages of handling. The number and types of bacteria in a shellfish product therefore reflects the changes that have occurred in the initial flora and the degree of contamination that has taken place in the course of handling on shore (Cann, 1977).

Detailed bacteriological evaluation of shrimp from cold waters have been attempted by Harrison and Lee (1969); Cann et al. (1971); Novak (1973) and Lee and Pfeifer (1977). Such data regarding tropical shrimp is rather scanty (Cann, 1977; Zuberi et al., 1983). The seafood industry needs a thorough knowledge of the bacteriology of processing operations so that adequate control measures can be taken or existing measures intensified at the required levels. This paper attempts to follow the major changes in the bacterial flora of prawns during preprocess handling, with emphasis on their quantitative as well as qualitative aspects.

2. MATERIALS AND METHODS

The tropical prawn Metapenaeus dobsoni was used throughout the study. The material which was collected from the fishing vessels operating from Cochin on its arrival at the landing places. It consisted of fresh, clean, wholesome prawns in the count range of 100-200/kg. The maximum time that could have elapsed between catch and collection of the sample was about 4 h. A portion of the material which was frozen whole, was iced immediately, while the rest of it was headed and iced. The prawn to ice ratio in both cases was 1:1. The material was then transported to the laboratory. The whole prawn and part of headed prawn were washed thoroughly with potable water carrying 5 ppm of chlorine and packed to be frozen as whole and headless-shell on'. The remaining part of the headed material was peeled, deveined, washed thoroughly and packed for freezing as peeled and deveined (PD). Generally the practices followed for handling and packing were as outlined in IS:4303 (Part I, 1977).

Bacteriological analysis of the sample was carried out by drawing the samples at five key points in the processing line. Thus representative samples were drawn from the fresh prawn immediately after arrival and collection, after heading, after icing for about 3 h, after peeling and deveining and after final washing and packing.

For bacteriological analysis the method followed in FDA (1978) was followed. For sampling, the meat was separated aseptically from the shell and homogenized with saline and serially diluted, and then 0.5 mL of the appropriate dilutions were spread plated on the sea water agar (SWA) and tryptone glucose agar (TGA) plates. Three sets of plates were used. One set was incubated for 21 days at $5 \pm 1^{\circ}C$ and the others for 2 days at room temperature (RT) ($20 \pm 2^{\circ}C$) and $37^{\circ}C$. Bacterial counts were recorded at the end of incubation period and the total plate count/g of the sample calculated.

For qualitative studies, about 30-50 colonies were picked randomly from SWA and TGA plates into tubes of sea water peptone (SWP) and tryptone glucose broth (TGB) respectively and incubated for 48 h. The colonies were purified by repeated streaking on SWA/TGA plates and maintained on slants of the same media for identification purpose.

For identification, the schemes proposed by Shewan et al., (1960); Lee and Pfeifer (1975) and Surendran (1980) were consulted in addition to Bergey's manual (Buchanan and Gibbons, 1974).

3. RESULTS AND DISCUSSION

Three series of experiments were conducted from three different batches of prawn collected during the period July-November, 1983. Data presented in Tables 1 and 2 represents the results of 3 typical series.

When plated on SWA, the total plate count of the freshly landed prawn ranged from 7.9×10^5/g to 3.1×10^6/g at RT. On TGA, the bacterial count fell within the range of 4.8×10^5/g to 1.1×10^6/g.

Heading the prawn lowered the bacterial count and the percentage reduction varied from 36.2 to 62.0. As seen from the data, icing had no significant impact on the total bacterial load. At room temperature incubation on SWA, there was an increase of 2.6% and 14.3% in two instances and a decrease of 11.4% in one instance. According to Castell et al., (1956), Georgala (1957) and Iyer and Chouduri (1966) icing introduced bacteria into fish, causing higher bacterial counts. Even though an increase was noted in this study, it was negligible. The exposure to ice was brief, extending only a few hours and that could be the reason for this behaviour.

Peeling and deveining resulted in an increase in bacterial count. The increase was found to be 32.4% to 65.5% based on the TGA count. On the other hand, on sea water based medium, the increased count was negligible. This demonstrates that the observed increase in TPC on TGA was due to terrestrial contaminants which failed to grow on sea water medium.

Washing resulted in a decrease in the total bacterial load, while the percentage of reduction due to washing was 50.54 to 80.71, on the basis of the count in SWA. According to Shewan (1971) most of the bacteria adhering to the slime and skin surface could be washed away with water.

The total plate count of the raw material reported in this study agreed well with that reported earlier. Shewan (1971) reported the bacterial count of shrimp to be in the range of 10^4-10^5/g, 10^5 being very common. Similar values were also reported by Harrison and Lee 1969); Hobbs et al. (1971); Baes et al. (1976) and Lee and Pfeifer (1977).

The bacterial count at $5 \pm 2^{\circ}C$ showed a downward trend. Gillespie and Macrae (1975) reported that the chlorinated water used for washing the fish caused a decrease of Gram negative psychrotrophs. Hence the observed decrease in bacterial count on plates incubated at $5 \pm 1^{\circ}C$ could be attributed to this.

The head portion of the shrimp constituted 40% of the body weight while it carried 75% of the total bacterial load (Novak, 1973). Hence the considerable decrease due to heading is understandable. The values reported in this study agreed with that reported by Williams et al. (1952) and Novak (1973). However, according to the results reported by Koburger et al., (1973) and Alvarez and Koburger (1978) no difference in bacterial count existed between whole and headless shrimp.

3.1 Qualitative aspects

The qualitative changes in the microbial flora of prawn Metapenaeus dobsoni during preprocess handling are given in Table 3.

The most striking feature of prawn processing was the enormous increase in Gram positives, mainly Micrococcaceae. This group which constituted less than 6% of bacteria on the raw prawn rose to 47% in peeled and deveined material. Harrison and Lee (1969) attributed the increase in Gram positives to increased handling by humans. A similar trend was noted by Lee and Pfeifer (1975) in crab processing.

The Moraxella - Acinetobacter group (formerly termed as Achromobacter) was the most predominant species among the Gram negatives, found on the raw shrimp. Even though they retained their number after heading, the subsequent steps i.e. icing, peeling and washing caused a fall. A similar trend was noted in the case of Vibrio and Flavobacterium spp. The Psuedomonas spp., which were almost equal to Vibrio spp., in population density, was found to increase after icing from 18 to 24%. However a drastic fall occurred after peeling.

Similar work reported from shrimp from cold water by Harrison and Lee (1969) and Lee and Pfeifer (1975). Those values are not quite comparable to the present study since their processes involved cooking and blanching. According to Lee and Pfeifer (1977) Pseudomonas increased from 17 to 44%, Moraxella was totally eliminated, while Acinetobacter and Flavobacterium increased. Also, the Micrococcus group formed a negligible portion of the bacteria on processed shrimps, but an increase in this group was noted in the present study and also in the study of Harrison and Lee (1969), with machine peeling being adopted in the former case, hand peeling was used in the latter.

It is clear from this study that washing and peeling are the two steps which determine the bacterial flora of the product. Even with extreme care, the overall reduction in the bacterial count that can happen to the raw material is less than one log order. Apart from the slight contamination in the peeling stage and partial removal of commensal bacteria during washing, the preprocess handling does not affect the bacterial quality of raw material considerably.

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Table 1

Quantitative changes in the microbial flora of prawn M. dobsoni during preprocess handling. Total plate count*/g.

Process	No. of trials	Bacterial count on SWA at 5±1°C	RT(29±2°C)	37°C	% increase/ decrease
Raw	1	1.04×10^5	2.11×10^6	1.37×10^6	
	2	2.00×10^5	3.13×10^6	2.86×10^5	-
	3	5.42×10^5	7.87×10^5	6.27×10^5	
Beheading	1	4.11×10^4	9.69×10^5	9.84×10^5	(-) 52.68
	2	7.80×10^4	1.18×10^6	9.58×10^5	(-) 62.01
	3	3.40×10^4	5.02×10^5	3.11×10^5	(-) 36.21
Icing	1	3.90×10^4	8.83×10^5	8.63×10^5	(-) 11.60
	2	8.20×10^4	1.22×10^6	1.28×10^6	(+) 2.60
	3	2.70×10^4	5.72×10^5	4.38×10^5	(+) 14.30
Peeling & deveining	1	3.10×10^4	7.97×10^5	7.37×10^5	(-) 9.73
	2	7.10×10^4	1.38×10^6	1.36×10^6	(+) 11.78
	3	2.20×10^4	6.87×10^5	6.91×10^5	(+) 20.10
Washing	1	1.30×10^3	1.21×10^5	1.25×10^5	(-) 80.71
	2	1.30×10^4	6.84×10^5	6.91×10^5	(-) 50.54
	3	3.20×10^3	2.11×10^5	3.42×10^5	(-) 69.28

* Average of 3 plates; (+) stands for increase in bacterial count
(-) stands for decrease in bacterial count

Table 2

Quantitative changes in the microbial flora of prawn M. dobsoni during preprocess handling. Total plate count*/g

Process	No. of trials	Bacterial count on TGA at 5±1°C	RT(29±2°C)	37°C	% increase/ decrease
Raw	1	1.37×10^{4}	6.24×10^{5}	3.83×10^{5}	
	2	5.80×10^{4}	1.08×10^{6}	1.05×10^{6}	-
	3	2.00×10^{4}	4.80×10^{5}	4.72×10^{5}	
Beheading	1	9.10×10^{3}	3.25×10^{5}	3.15×10^{5}	(-) 48.02
	2	3.60×10^{4}	6.23×10^{5}	6.08×10^{5}	(-) 42.30
	3	8.21×10^{3}	2.13×10^{5}	2.08×10^{5}	(-) 55.63
Icing	1	8.03×10^{3}	3.10×10^{5}	3.20×10^{5}	(-) 4.62
	2	3.40×10^{4}	6.35×10^{5}	6.36×10^{5}	(+) 1.92
	3	8.47×10^{3}	2.28×10^{5}	2.01×10^{5}	(+) 7.00
Peeling & deveining	1	4.90×10^{3}	5.13×10^{5}	5.27×10^{5}	(+) 65.48
	2	3.10×10^{4}	9.40×10^{5}	9.58×10^{5}	(+) 32.44
	3	1.08×10^{4}	3.34×10^{5}	3.59×10^{5}	(+) 46.49
Washing	1	0.13×10^{3}	1.33×10^{5}	1.44×10^{5}	(-) 74.07
	2	2.60×10^{3}	2.51×10^{5}	2.59×10^{5}	(-) 73.22
	3	0.88×10^{3}	1.08×10^{5}	1.23×10^{5}	(-) 67.66

* Average of 3 plates; (+) stands for increase in bacterial count
(-) stands for decrease in bacterial count

Table 3.[1] Qualitative changes in the distribution of major microbial group in *M. dobsoni* during preprocess handling

Bacterial genus	Raw			Beheaded			Iced			Peeled & deveined			Whole			Washed and packed Headless			PD		
	Temperature of incubation (°C)																				
	5±1	RT	37	5±1	RT	37	5±1	RT	37	5±1	RT	37	5±1	RT	37	5±1	RT	37	5±1	RT	37
Pseudo_ monas	22*	17	12	20	18	14	24	23	11	11	7	21	20	20	23	23	24	6	6	6	5
Moraxella	22	20	18	21	18	17	20	21	19	8	12	11	17	16	18	13	15	10	10	10	11
Acineto- bacter	18	19	20	19	21	20	19	18	18	12	11	12	16	16	17	16	17	14	12	12	12
Vibrio	15	18	22	16	18	23	14	15	15	12	12	14	12	14	14	14	15	15	13	14	13
Flavo bacterium/ cytophage	10	9	10	8	9	10	18	6	10	6	4	4	8	6	3	3	5	4	6	4	4
Entero- bacteri- aceae	0	0	0	0	0	6	0	0	1	0	2	2	0	1	1	0	0	1	0	0	2
Micro co- ccaceae	5	6	7	3	5	0	3	4	4	39	40	40	19	20	25	20	23	24	46	47	47
Bacillus	0	1	1	2	1	2	1	2	1	4	2	3	3	2	2	2	1	1	3	3	2
Arthro- bacter	8	10	10	11	10	8	1	10	9	8	6	7	4	4	2	4	4	2	4	4	4
Total isolates	72	83	79	81	88	70	88	76	81	70	68	82	73	78	77	85	83	83	68	80	80

* Expressed as percentage of total isolates

INCIDENCE OF HUMAN PATHOGENIC VIBRIOS IN SEAFOODS HARVESTED ALONG THE COAST OF KARNATAKA (INDIA)

by

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ABSTRACT

The incidence of human pathogenic vibrios in seafoods harvested along the coast of Karnataka State (India) was studied by enrichment and direct plating methods. Vibrio parahaemolyticus appeared to be the most common (69% incidence); non-01 V. cholerae were observed even in samples taken on board the fishing vessels. V. vulnificus and V. mimicus were other human pathogens observed. There appeared to be no correlation between faecal coliform count and incidence of human pathogenic vibrios suggesting that the latter are native to the aquatic environment.

INTRODUCTION

During recent years, a number of Vibrio species have been shown to be associated with food-borne diseases in man. Vibrio cholerae 01, the well known causative agent of cholera has been reported to be present even in waters free from faecal contamination (Colwell, 1984). V. parahaemolyticus was the first of the non-cholera vibrios to be widely recognised as a serious human pathogen. Vibrios that are biochemically very similar to the epidemic strains of V. cholerae (V. cholerae 01) but that do not agglutinate in V. cholerae 0 group 1 anti-serum have been termed non 01 V. cholerae. These have been found to be associated with diarrhoea (Zafari et al. 1973 and Dakin et al. 1974). V. vulnificus has been reported to cause two distinct clinical syndromes in infected persons; primary septicaemia and wound infection (Blake et al. 1979). Raw oyster ingestion has been suggested as the source of infection for patients with primary septicaemia (Johnston, Becker and McFarland, 1985). V. mimicus has been isolated from shellfish and water as well as from human diarrhoea stool and ear infections (Davis et al. 1981). In this communication, we report the incidence of some of these human pathogenic vibrios in seafoods harvested around Mangalore, West coast of India.

MATERIALS AND METHODS

Samples of shrimp, fish and molluscan shellfish were collected from shrimp processing factories, fish landing centres and fish markets. Some samples were collected on board the fishing vessel and transferred to sterile containers. All samples were transported to the laboratory within an hour of collection and analysed immediately.

For isolation of V. cholerae, the methodology outlined in FDA Manual (FDA, 1978) was used. Samples of 50 g were homogenised with 450 mL sterile alkaline peptone water. Homogenates were transferred to sterile 1 L Erlenmeyer flasks and incubated at 37°C for 6 hours, after which a 5 mm loopful was streaked on thiosulphate citrate bile sale sucrose (TCBS) agar and incubated for 24 hours at 37°C. About 10 large smooth yellow colonies characteristic of V. cholerae were picked up and purified on Tryptic soy agar (TSA). Biochemical tests described in the FDA Manual were carried out and those colonies showing typical V. cholerae characteristics were studied for agglutination with V. cholerae Ogawa and Inaba antiserum obtained from the Central Research Institute, Kasauli.

Sucrose negative bluish green colonies on TCBS were also purified (10 from each sample on TSA and tested for biochemical reactions typical of V. parahaemolyticus, V. vulnificus and V. mimicus. V. parahaemolyticus strains were tested for Kanagawa reactivity on Wagatsuma agar (Hi Media, Bombay).

Levels of V. parahaemolyticus in shrimp was assessed using the most probably number (MPN) method with glucose salt teepol broth (GSTB) as described in the FDA Manual. Faecal coliform count was also performed by MPN method described in the FDA Manual.

RESULTS AND DISCUSSION

Results in Table 1 show that V. parahaemolyticus was the most common (69%) vibrio found in all the samples tested. V. vulnificus was present in 25% of samples, V. cholerae in 9% and V. mimicus in 6.5% of the samples. None of the V. cholerae isolates sgglutinated with 01 antiserum. None of the V. cholerae strains have been reported to be widely distributed in the environment in Europe, Asia and United States. Blake et al.(1984) surmised that V. cholerae is an autochithonous species of the estuarine ecosystem inasmuch as the occurrence of V. cholerae was not correlated with other microbial indicators of faecal pollution. Even in this study, we did not find statistically significant correlation between faecal coliform count and incidence of V. cholerae (Table 3). Presently, non 01 V. cholerae are not included in microbiological standfards for seafoods. However, this organism has been recognised as the causative agent of sporadic outbreaks of gastroenteritis. Craig et al. (1981) reported that certain non 01 V. cholerae strains produce a toxin that is identical to cholerae enterotoxin but Yamamoto et al. (1983) showed that such strains produce a toxin that is similar but not identical to cholerae enterotoxin. Arita et al. (1986) purified and characteristed a heat stable enterotoxin produced by non 01 V. cholerae. There is a need to assess the toxigenicity of strains isolated from seafoods.

Though there are no records of rejection of shrimp in international trade due to presence of V. parahaemolyticus, the International Commission for Micriobiological Specifications for Foods (ICMSF, 1974) has suggested a safety limit for this organism in frozen shrimp and lobster tails, frozen cooked shrimp and lobster tails, frozen raw and breaded shrimp and cooked picket crab meat. It had earlier been reported that shrimp processors around Mangalore are able to meet the ICMSF Specification of 10^2 V. parahaemolyticus per gram of the sample (Karunasagar et c. 1984). Results in Table 2 also show that Most Probable Number (MPN) of V. parahaemolyticus in frozen shrimp was always less than 10/g. A small number (9-12%) of raw and processed shrimp had counts in the range 10^1-10^2/g. Results in Table 3 show that there was no correlation between incidence of V. parahaemolyticus and faecal coliform levels. Kaneko and Colwell (1973) also did not find correlation between Escherichia coli and V. parahaemolyticus counts in Chesapeake Bay. Most of the strains of V. parahaemolyticus isolated from cases of gastroenteritis have found to produce haemolysis in a special blood agar medium, Wagatsuma agar (Sakazaki et al. 1968). We have earlier reported that a small percentage of environmental strains around Mangalore are haemolytic (Karunasagar and Mohankumar, 1980) and further studies (Karunasagar, 1985) indicated that the incidence of these haemolytic strains do not correlate with faecal coliform levels.

V. volnificus and V. mimicus are not part of the microbiological standards for seafoods at present. However, US FDA warned that V. vulnificus can get into the human body through cuts or by eating contaminated oysters. Like other human pathogenic vibrios discussed here, V. vulnificus and V. mimicus also appear to be native to the aquatic environment inasmuch as their incidence was not related to faecal coliform levels (Table 3). Oliver et al. (1983) documented lack of relation between incidence of V. vulnificus and faecal contamination of waters. Bockemuhl et al. (1986) reported that incidence of V. mimicus was not influenced by the number of coliforms or faecal coliforms. Strains of V. mimicus have been shown to produce heat labile and stable enterotoxins (Davis et al. 1981) and therefore, there is a need to investigate the toxigenicity of V. mimicus strains associated with seafoods.

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Table 1. Incidence of human pathogenic vibrios in various samples

Sample	Total no.	No. positive for *V.cholera*	*V.parahae-molyticus*	*V.vulnificus*	*V.mimicus*
Prawns	71	6	57	20	1
Fish	86	12	50	16	9
clams	26	Nil	18	7	3
Oysters	15	Nil	12	6	Nil
Mussels	2	Nil	1	1	Nil
Total	200	18	138	50	13

Table 2. MPN of *V. parahaemolyticus* in shrimp from processing factories around Mangalore

Sample	No.	No. of samples showing MPN/g		
		0.2-10	$10-10^2$	$>10^2$
Raw	56	20	5	-
Processed	50	18	6	-
Frozen:				
PUD	28	-	-	-
Headless	29	3	-	-

Table 3. Correlation between faecal coliform levels and human pathogenic vibrios in various samples

Sample	Correlation coefficient			
	V.cholera	*V.parahaemolyticus*	*V.vulnificus*	*V.mimicus*
Prawn	0.152	0.217	-0.091	0.092
Fish	0.094	0.145	0.148	-0.126
Bivalves	No isolates	0.034	0.058	0.099

None of these are significant at 5% level

MICROBIOLOGICAL CHANGES IN PRAWN (PENAEUS SP) DURING PROCESSING IN SRI LANKA

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ABSTRACT

Microbiological evaluation of prawn processing was made once a month, from samples obtained at four processing stages in a factory, over a period of six months. The bacterial counts of raw material ranged from 10^5 to 10^8/g with 45% of the samples in the 10^6 to 10^7/g range. The reduction of the bacterial load in the end product (frozen) was significant with 58% samples in the 10^5 to 10^6/g range. Escherichia coli counts in the raw material ranged from <3 to $>10^3$/g but were reduced to between <3/g (66.7%) and 3 to 10^2/g (33.3%). Staphylococcus aureus ranging from <50/g to 10^3/g in the raw material increased to 10^5 to $>10^6$/g during processing. More than 65% of the workers in the factory were found to carry S. aureus on their fingers. The S. aureus levels reduced significantly at the stages just before freezing and after freezing, with almost all samples with counts <50/g. Vibrio parahaemolyticus was detected only in the raw material (23/g and 4/g) on two occasions, and was not found in other stages of processing. Salmonella was not detected. The tap water in the processing area of the factory was found to be contaminated with coliforms on four occasions and with E. coli on one occasion. The temperature of the prawns along the process line varied from 1^oC to 19^oC, with the highest temperature being recorded just before freezing. The initial microflora in order of predominance was Micrococcus, Flavobacterium, Bacillus, Coryneforms, Pseudomonas and Aeromonas. Coryneforms, Pseudomonas and Flavobacterium increased slightly during processing, but in the final product gram-positive, Micrococcus, Bacillus and Coryneforms were respectively predominant.

1. INTRODUCTION

Sri Lanka exports marine products valued at approximately $15 million, annually. Crustaceans represent nearly 75% of these exports. The main crustacean exported is block frozen prawn. The major export markets for these products are Japan and the United States of America.

There are many types of frozen prawn products and of these, the following constitute the main types of products exported from Sri Lanka ;

- whole, shell on, raw, frozen,
- headless, shell on, raw, frozen,
- headless, cooked, peeled, frozen,
- peeled, undeveined, raw, frozen (PUD),
- peeled, deveined, raw, frozen (P&D).

All processing prior to freezing is carried out manually.

The number and type of bacteria on a crustacean product reflect the initial bacterial load as well as the changes that have taken place during harvesting, handling and processing. Crustaceans caught in deeper waters usually carry only marine bacteria but those caught in inshore waters may carry terrestrial types as well. As contamination occurs as a result of contact with man during harvesting and handling on board, fresh crustaceans may be contaminated with common food-borne pathogens.

Literature on the bacterial content of crustaceans collected directly from the sea is scarce, and the data available is only for freshly landed crustaceans. The bacterial counts and flora of tropical crustaceans differ from cold water species, the former being largely mesophilic, due to the higher environmental temperatures. Pillai et al., (1961), Jacob et al. (1962), Cann (1974), Sumner et al. 1982), Fonseka (1985), Surendran et al., (1985) have worked on crustaceans caught in tropical waters.

The successful development of a crustacean industry depends not only on the improved supply of raw material but also on the quality of exports. In this respect attempts should be made to satisfy bacteriological specifications, limits and standards laid down by the importing countries. On the other hand realistic appraisal of the practicability of attaining these bacteriological requirements must be made. A thorough knowledge of the bacteriology of the specific variety of crustaceans needs to be obtained at all stages of catching, handling and processing. The present study was undertaken to collect the necessary background information on the quality and quantity of microflora in our prawn industry.

2. MATERIALS AND METHODS

Prawn (Penaeus spp) samples were collected along the processing line, from the processing factory (Fig. 1). A total of 14 samples were collected from raw material (4), processing line (4), before freezing (4) and frozen product (2). Samples were collected using sterile forceps, into sterile polythene bags, sealed and stored in an insulated box with ice and transported to the laboratory. These samples were examined the same day, for total bacterial count (TBC) 30°C, E. coli count (MPN), Staphylococcus aureus, Vibrio parahaemolyticus (MPN) and Salmonella (AOAC, 1978). Bacterial colonies on nutrient agar plates were picked out randomly, purified and morphologically and biochemically characterised and classified (Buchanan, 1974, Shewan et al., 1960, Cowan and Steel, 1974 and Lee and Pfeifer, 1975). This sampling was repeated on six occasions, at the same factory.

Four samples of tap water were collected into sterile bottles containing 0.1 mL 10% $Na_2S_2O_3$. These samples were examined for total plate counts, total Coliforms and E. coli.

On all sampling occasions the finger impression of the workers handling prawn were taken on Baird-Parker agar to test for the presence of S. aureus, on their hands.

3. RESULTS AND DISCUSSION

The flow chart of prawn processing is presented in Fig. 1, which also indicates the points at which the samples were drawn. The product on the processing line which was sampled was headless, shell on, raw prawns.

The total counts of raw material (Table 1) were from 1.4×10^5/g to 2.6×10^7/g, with a majority of samples in the 10^6-10^7/g (45.8%) and 10^5-10^6/g (37.5%) range. Sumner et al. (1982) found total bacterial counts ranging from 10^5-10^8/g for raw material used in prawn processing in Sri Lanka. Foseka (1985) reported counts of 10^6/g for freshly landed prawns, caught in Negombo, Sri Lanka. Bacterial counts reported for prawns in India were from 10^4 to 10^7/g (Pillai et al., 1961 and Jacob et al., 1962). Surendran et al.(1985) reported that the initial bacterial load ranged from 10^3-10^4/g for prawns collected in sterile containers from the trawlers, thus avoiding normal handling practices. In the present study the bacterial count on prawns (raw material) at the factory was generally high (>10^6/g) but it seems to be the natural level for tropical prawns which has undergone normal handling on board, at landing and during transportation to the factory, as similar levels have been reported by other workers from tropical areas. The raw material was deheaded and graded on the processing line. Deheading is reported to reduce the bacterial counts by about 75%, as most of the bacteria were found in the cephalothorax (Novak, 1973). Cann (1977) on the other hand,states that based on studies in Thailand and USA, the difference in bacterial counts between whole and deheaded prawns was not significant. The deheading of prawns in the present study was carried out manually. Although the percentage of bacterial counts in 10^6-10^7/g range showed an increase to 62.5%, from an initial 45.8%, the statistical analysis of the difference in total bacterial counts between whole (raw material) and deheaded (processing line) prawn was found to be insignificant. At the next stage of processing (before freezing), the processed prawns were washed two or three times in clean water prior to packing in trays. The workers engaged at this point were wearing rubber gloves. The effect of washing on the bacterial load of the processed product was evident as a majority (58.3%) of counts fell within the 10^5-10^6/g range, and this reduction was statistically significant. During processing bacterial numbers and types fluctuate with counts rising during sorting, peeling, grading and packing and falling at washing, cooking and freezing stages (Cann, 1977). The final product (after freezing) had 58.3% counts in the 10^5-10^6/g range and only 16.6% in the 10^6-10^7/g range. The total bacterial count in all samples of frozen prawns fall within the limits of ICMSF and Sri Lankan Standards (Table 9).

The E. coli counts of raw material range from <3/g to 1,100/g, with 50% of the counts in the 3-10^2/g range. Prawns caught in deeper waters generally do not carry E. coli, but those caught close to the shore and from inshore waters have been found to be contaminated frequently with E. coli (Cann, 1977). The handling on board and transportation, adds to the contamination. The E. coli counts do not vary significantly during the processing stage of deheading and grading, but the count declines slightly during the next stage, just before freezing, and is significantly reduced after freezing, with 66.7% in the <3/g and 33.3% in the 3-10^2/g range.

The Staphylococcus aureus counts are presented in Table 3, and the counts in the raw material were mainly in the <50/g (70.8%) range. A few samples contained counts in the 50-100/g and 10^2-10^3/g ranges. However, during the process of deheading and grading the S. aureus counts increased significantly. The factory provides uniforms and head coverings to all personnel and sanitiser hand dips. It appears that the trend is to move away from the use of sanitiser hand dips, because some are not lethal for S. aureus and also they may facilitate the build up of S. aureus by eliminating harmless competing bacteria on the hands (Sumner et al., 1982). The workers at this factory do not wear gloves at this stage of processing as it hinders speed and efficiency in deheading and peeling. The presence of S. aureus on the hands of workers in the processing plant is presented in Table 4. Except on one occasion the test records over 65% of workers to be carrying S. aureus on their hands. The carrier rate of S. aureus among healthy adults has been reported to be 30-50%, the organism being carried on the hands, ears, nose and throat (Eyles, 1976).

The workers engaged in the next stage of processing, which was washing prior to packing in trays, wore rubber gloves. The processed prawns were washed thoroughly in clean water 20 or 3 times. The effect of thorough washing and use of gloves was evident in the S. aureus level as all samples had counts less than 50/g. The frozen product also had very low S. aureus counts (91%-<50/g). The presence of S. aureus in large numbers indicate poor personal and plant hygiene and improper sanitary procedures in a processing plant. The S. aureus counts on the samples during this study were all within the limits of ICMSF and Sri Lankan Standards.

Vibrio parahaemolyticus is naturally found in sea water, and has been found to cause food poisoning in individuals consuming uncooked or lightly cooked seafoods during warm periods of the year especially in cold/temperate climates, which points to two characteristics of this organism; halophilism and sensitivity to both heat and cold (Johnson and Liston, 1973). V. parahaemolyticus was detected on 2 out of the 6 sampling occasions, and only in one each of the 4 sub-samples of raw material (Table 5). It occurred in very low numbers, 23/g and 4/g during the present study, but this organism was not detected at the other stages of processing. Karunasagar and Venugopal (1984) had also found low levels of V. parahaemolyticus in Indian shrimp undergoing processing for export. Most samples contained <10/g and a few between 10-10^2/g, and no samples had counts of >10^2/g which was the proposed ICMSF limit. This organism is highly sensitive to temperatures in the range 0-5°C, and chilling is thought to be an effective method of control except when initial contamination is very high. Freezing, though less lethal than chilling is also an effective method of control (Liston, 1976). As the results from this study indicated V. parahaemolyticus does not present a problem for the Sri Lanka prawn industry.

Salmonella sp. which cause infections such as gastroenteritis and enteric fever in man, is an organism which is examined for by most importing countries and is expected to be absent from a sample of 25 g (Table 9). During the present study Salmonella was not detected in 25 g samples from any stage of processing.

The bacterial quality of the water used in the processing of prawn is given in Table 6. The total bacterial count was <10/100 mL in 60% of the samples examined, and the rest had counts between 10-100/100 mL (20%) and 10^2-10^3/100 mL (20%). The Coliform level for potable water should be less than 10/100 mL and 58% of the water samples examined had coliform levels of <10/100 mL, while the rest had higher counts for Coliforms and one sample had a count of 9.2×10^2/100 mL, which was very high. The same sample had an E. coli, count of 5/100 mL while E. coli was not detected in the other samples.

Control of prawn temperature during processing is important. Maintaining a temperature below 10°C is considered adequate, provided that the processing time is not prolonged (Sumner et al., 1982). The temperatures of prawns, in the present study is given in Table 7. The temperature of raw material at the receiving point was found to be below 10°C, except on one occasion. The temperature control was found to be inadequate on 2 sampling occasions (4th and 5th), and on almost every occasion the temperature of the samples just before freezing was high, and control inadequate on 3 occasions. The icing of prawns during processing (deheading and grading) was satisfactory, as they used crushed ice in adequate quantitites. The reason for the higher temperatures at the next stage was the final washing of prawns in water of 28-30°C, during the first 3 occasions of the study. During the period of the study the factory installed a chill water facility, and this water was used for the final washing stage, which improved the temperature control at this stage of processing.

The major groups of bacteria isolated from the prawns sampled in this study are presented in Table 8 and at all stages of sampling gram positive organisms dominated, comprising 54%, 61%, 56% and 63% of raw material, processing line, before freezing and frozen product, respectively. Of the gram positive bacterial, Micrococcus predominated at all stages. In the raw material, Bacillus, Flavobacteria and Coryneforms followed, in order of occurrence, and Aeromonas, Pseudomonas, Vibrio, Moraxella and Staphylococcus were also isolated. The bacterial flora of tropical species of crustacea differs from that of cold water species. The flora on shrimps from cold or temperatre waters consist largely of either Achromobacter (Acinetobacter - Moraxella group) or Coryneforms.

Pseudomonas and Micrococcus were significantly present (Cann, 1976). According to Sreenivasan (1959) Indian prawns carried a predominantly micrococcal and corynebacterial flora. Cann (1974) reported that the dominant genera on prawns in Thailand to be Micrococcus and Coryneform, and to a lesser extent Flavobacterium and Achromobacter; and for prawns from Malaysia the major types were Micrococcus and Achromobacter, with Coryneform and Flavobacterium occurring to a lesser extent. Fonseka (1985), reported that the majority of organisms isolated from prawns in Sri Lanka to be gram positive and predominantly Micrococcus and Bacillus.

During the stages of deheading, grading and washing prior to freezing there is hardly any change in the major bacterial species. There is a slight increase in the occurrence of Pseudomonas and Flavobacteria. In the final product (frozen) Micrococcus, Bacillus and Coryneforms predominated respectively. The spoilage flora associated with iced seafood belong to the gram negative group of bacteria. Pseudomonas and Moraxella - Acinetobacter species were reported to be the dominant flora in the spoilage of tropical prawns in ice (Cann, 1974, Surendran and Gopakumar, 1982). The total counts of the final product was mainly in the 10^5-10^6/g range and was within the recommended limits. Considering the composition of the flora it seems very unlikely that this would pose a threat of spoilage during storage at low temperatures.

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Table 1

Total bacterial count of prawns along the processing line

Range (/g)	10^4-10^5	10^5-10^6	10^6-10^7	10^7-10^8
Raw material	-	37.5[a](9)[b]	45.8(11)	16.6(4)
Processing line	4.2(1)	20.8(5)	62.5(15)	12.5(3)
Before freezing	-	58.3(14)	33.3(8)	-
Frozen	25.0(3)	58.3(7)	16.6(2)	-

a - percentage of samples
b - number of samples

Table 2

Escherichia coli (MPN) count of prawns along the processing line

Range (g)	<3	3-10^2	10^2-10^3	10^3-10^4
Raw material	8.3[a](2)[b]	50.0(12)	29.0(7)	12.5(3)
Processing line	12.5(3)	54.1(13)	16.7(4)	16.7(4)
Before freezing	20.8(5)	62.5(15)	12.5(3)	4.29(1)
Frozen	66.7(8)	33.3(4)	-	-

a - percentage of samples
b - number of samples

Table 3

Staphylococcus aureus count of prawns along the processing line

Range (/g)	<50	50-102	10^2-10^3	10^3-10^4	>10^4
Raw material	70.8[a](17)[b]	16.7(4)	12.5(3)	-	-
Processing line	41.7(10)	12.5(3)	33.3(8)	4.2(1)	8.3(2)
Before freezing	100.0(24)	-	-	-	-
Frozen	91.7(11)	-	8.3(1)	-	-

a - percentage of samples
b - number of samples

Table 4

Incidence of Staphylococcus aureus on the hands of the workers in the processing plant

Sampling occasion	No. of workers examined	No. of workers positive for S.aureus	Percentage of S.aureus carriers
1	17	15	88.2
2	20	14	70.0
3	08	01	12.5
4	27	18	66.6
5	15	12	80.0
6	15	10	66.6

Table 5

The occurrence of V. parahaemolyticus in prawn during processing

Sampling occasion	1	2	3	4	5	6
Raw material	<3	<3	<3	<3	<3	<3
	<3	-	-	<3	-	-
	<3	-	-	4	-	-
	23	-	-	<3	-	-
Processing line			NOT	DETECTED		
Before freezing			NOT	DETECTED		
Frozen			NOT	DETECTED		

Table 6

The bacterial counts of water used in the processing of Penaeus sp.

Range	<2	<10	10-100	10^2-10^3
TPC/mL	01	2(60)	4(20)	4(20)
Coliforms	13(54.2)	1(4.2)	9(37.5)	1(4.2)/100 mL
E. coli	23(95.8)	1(4.2)	0	0/100 mL

Table 7

Temperature (^{o}C) control of prawns during processing

Sampling occasion	1	2	3	4	5	6
Raw material	5-9	1-5	1-5	5-10	1-5	3-18
Processing line	3-8	1-5	3-5	2-17	9-14	1-5
Before freezing	8-19	5-11	7-12	5-10	5-8	5-10

Table 8. The percentage occurrence of the major bacterial genera found on prawns during processing

		Micrococcus	Staphylococcus	Coryneform	Bacillus	Vibrio	Aeromonas	Pseudomonas	Flavobacteria	Moraxella	Acinetobacter	Enterobacteriaceae
Raw material	(1)	30	-	35	5	-	10	20	-	-	-	-
	(2)	50	5	-	5	-	20	10	5	-	5	-
	(3)	25	10	10	-	-	-	-	30	-	-	-
	(4)	50	-	-	-	20	-	-	15	15	-	-
	(5)	25	5	-	25	5	15	-	15	-	-	5
	(6)	5	-	10	30	10	-	15	-	10	-	10
Average		30.8	3.3	9.1	10.8	5.8	7.5	7.5	10.8	4.2	0.8	2.5
Processing line	(1)	45	-	20	15	-	5	15	-	-	-	-
	(2)	30	5	15	20	-	5	10	10	-	-	-
	(3)	10	5	15	-	5	-	25	10	10	5	-
	(4)	65	-	-	10	-	-	-	25	-	-	-
	(5)	20	10	25	-	-	5	10	15	10	-	-
	(6)	20	10	25	-	-	5	10	15	10	-	-
Average		31.7	5.0	16.7	7.5	0.8	3.3	11.7	12.5	5.0	0.8	-
Before freezing	(1)	20	-	10	35	-	10	15	-	-	-	-
	(2)	30	15	10	5	-	-	20	15	-	-	-
	(3)	20	-	25	5	-	-	15	10	-	-	5
	(4)	45	-	-	15	15	-	-	25	-	-	-
	(5)	10	10	25	15	-	10	5	20	5	10	-
	(6)	10	10	25	5	-	10	5	20	5	10	-
Average		22.5	5.8	15.8	11.7	2.5	5.0	10.0	15.0	1.7	3.3	0.8
Frozen	(1)	20	-	5	60	-	10	5	-	-	-	-
	(2)	30	-	30	10	-	-	5	10	-	5	-
	(3)	40	-	-	-	30	-	-	-	-	-	-
	(4)	25	-	-	40	5	-	15	15	-	-	-
	(5)	25	-	35	-	-	-	-	-	35	-	-
	(6)	25	-	35	-	-	-	-	-	-	35	-
Average		27.5	-	17.5	18.3	5.8	1.7	4.2	4.2	5.8	6.7	-

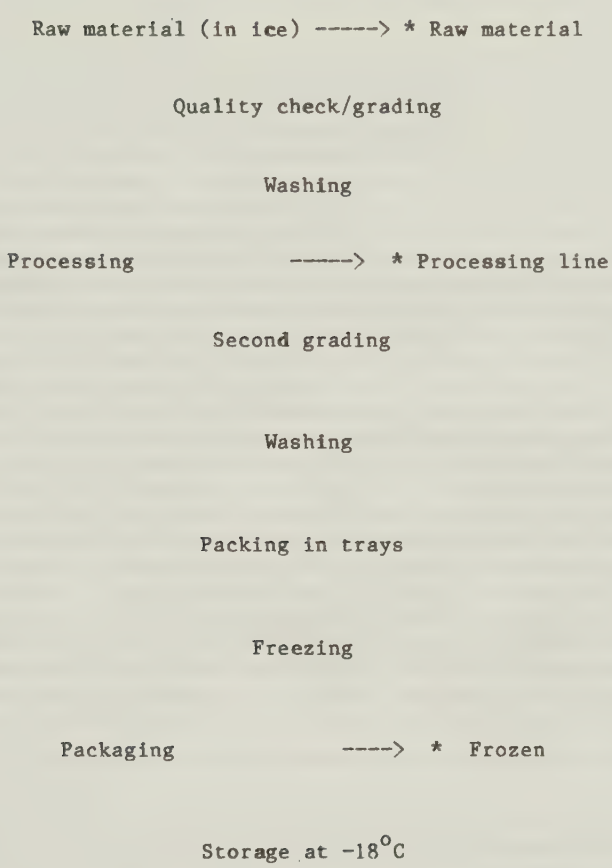
Table 9

Some bacteriological standards and limits for frozen raw prawns and lobster tails

Test	Sample number	Tolerance m-M	Limits/g m	M
ICMSF - TPC(/g)	5	3	10^6	10^7
Faecal coliforms (MPN/g)	5	3	4	400
Staph. aureus (/g)	5	3	10^3	$2x10^3$
Vibrio parahaemolyticus (/g)	5	0	-	10^2
Salmonella (/25g)	5	0	-	Nil
Sri Lanka - TPC (/g)	5	3	10^6	10^7
Faecal coliforms (MPN/g)	5	3	20	400
Staph. aureus (/g)	5	3	500	5000
Salmonella (/25g)	5	0	-	Nil

Figure 1

Flow Chart of Prawn Processing



SOME CHEMICAL AND MICROBIOLOGICAL CHANGES DURING CHILLED STORAGE OF PRAWNS (PENEAUS INDICUS)

by

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ABSTRACT

Prawns iced approximately eight hours after capture remained acceptable organoleptically (cooked) for 12 days. Raw prawns were judged to be Grade I up to three days, Grade II on the seventh day and were rejected on the 10th day in ice. The pH of the prawns was 7.31 initially and increased to 7.94 during storage and was 7.86 at rejection. The total volatile nitrogen (TVN) level decreased during the first week and increased thereafter from 2.7 mg N/100 g to 49.3 mgN/100 g, on the 14th day in ice, and was 44 mg/100 g N at rejection. The trimethyl amine content was 1.72 mg/100 g N initially and increased to 5.08 mg/100 g N on the 17th day. This increase was too low to be of value as a quality index. The TVN/AA-N (alpha-amino nitrogen) ratio obtained from this study was >1.2 mg N/mM on the first day and remained at <1.5 mg N/mM up to the 14th day in ice. The level of indole was very low up to seven days of storage (<2.5 mg/100 g) and thereafter it increased to reach 22.7 mg/100 g on the 10th day and 44.8 mg/100 g on the 14th day in ice. Of the chemical parameters analysed pH, TVN and indole correlated with taste panel scores and were found to be useful as indicators of quality. The total bacterial count was 3.0×10^5/g on the first day in ice and 1.8×10^8/g on the 14th day. The initial microflora consisted of Aeromonas (27%), Micrococcus (26%), Staphylococcus (21%), Pseudomonas (19%) and Enterobacteriaceae (6%). Moraxella, which was not isolated initially, comprised 10% of the isolates on the seventh day and 40% on the 17th day at spoilage. The dominant flora of the spoilt prawns were Aeromonas and Moraxella spp.

1. INTRODUCTION

The easiest and most common method of preserving fish and shellfish in the fresh form, is by icing. In the Sri Lankan prawn industry, prawns are kept in ice during transportation to factories, storage and processing.

Prawns contain high levels of free amino acids which are responsible for their characteristic flavour, which also facilitiates rapid bacterial growth. Deterioration of quality in prawns is considered generally to be due to the action of enzymes from both the tissue and the microorganisms present on the prawns or introduced during harvesting and handling. Spoilage of prawns is mainly due to bacterial action which forms compounds imparting off-odours, off-flavours and colour changes.

Prawns die soon after catching, thus differing from other crustaceans such as lobsters and crabs. Deterioration consequently, starts earlier. The most prevalent method of preserving prawns in the fresh form is by using ice. The formation of black spots is due to melanosis occurring during the storage of prawns. Though this is not connected directly with spoilage, it gives a poor appearance to the prawns and makes them unacceptable to the consumer.

The storage of prawns in ice has been studied by many workers, (Velankar et al., 1961, Farooqui et al., 1978, Foda et al., 1984, Surendran et al., 1985, Matches, 1982, Flores and Crawford, 1973) and the deterioration in quality has been followed by taste panel assessment, total volatile nitrogen (TVN), trimethylamine (TMA), alpha amino nitrogen (AAN), pH, orthophosphate and non-protein nitrogen.

During storage in ice, even under the best conditions, bacteria increased after a lag phase of about 2 days, reaching a maximum of about 10^7 to 10^8/g after 9 to 10 days (Shewan, 1961). During this period the bacteria change in numbers as well as the types.

The changes in the bacterial flora of prawns has been studied previously, but not as extensively as that of fish. Campbell and Williams (1952) studied the changes in the bacterial flora of Mexico shrimp, Cann (1974) working on prawns from the Gulf of Thailand reported a shift in

the microflora from predominantly gram positive initially, to mainly Pseudomonas spp. during storage in ice. Matches (1982), Surendran and Gopakumar (1981 and 1985), Wu and Chen (1980) and Reilly et al., (1986), have also studied the changes in the microbial flora of prawns during ice storage.

This study on the ice storage of prawns was carried out to determine the storage life and to osberve some aspects of chemical and microbiological changes during storage and as such, quality deterioration was measured by taste panel assessment, TVN, TMA, pH, AAN, Indole and Total Bacterial Counts.

2. MATERIALS AND METHODS

Prawns (Penaeus sp.) were collected from the landing centre, Chilaw, mixed with ice (1:1 ratio), and transported to the laboratory in an insulated box. At the laboratory the prawns were re-iced and stored in the wet fish cabinet, pre-set at 0° to $+5^{\circ}C$. Samples were drawn on the 1st, 3rd, 7th, 10th, 14th and 17th day of storage and changes in quality was followed by organoleptically and chemically.

2.1 Organoleptic Examination

Raw appearance was observed and the same prawns cooked and judged by a trained panel for odour, flavour, texture and overall quality using a hedonic scale of 10-0, where the limit of acceptability is 4.

2.2 Total Volatile Nitrogen/Trimethylamine Content

Samples analysed according to the Conway-Byrnes method (modified method of Beatty and Gibbons, 1937).

2.3 Indole Content

Indole was determined according to AOAC method 18.072 (Horwitz, 1980).

2.4 Amino Nitrogen Analysis

Amine nitrogen was analysed by a modification of the copper procedure of Spies and Chambers (1951), as described by Cobb et al., (1973).

2.5 Measurement of pH

The pH was measured by Radiometer Type PHM 26.

2.6 Microbiological Analysis

Total viable counts were estimated on the 1st, 3rd, 7th, 10th, 14th and 17th day of storage and the cultures were isolated from TPC plates on the 1st, 7th, 14th and 17th day of storage. These were morphologically and biochemically characterised and classified using Buchanan and Gibbons (1974), Shewan et al., (1960), Cowan and Steel (1977) and Lee and Pfeifer (1975).

3. RESULTS AND DISCUSSION

The taste panel scores of cooked prawns are presented in Figure 1. The score on the 1st day of storage were 8.6 declining to 6.3 on the 10th day. On the 14th day the prawns were judged to be unacceptable (score 4.0), on the 12th day in ice. As judged by the raw appearance (Table 1) the prawns could be classed as Grade I on the 1st and 3rd days in ice, Grade II on the 7th day and just acceptable on the 10th day, after which they were rejected. Farooqui et al. (1978) reported that prawns in ice maintained good quality for 0-2 days as judged by raw appearance; was acceptable up to 7 days and rejected after 9 days. According to Cann (1974), prawns in ice start to lose flavour early (2-4 days), black spots are formed by 6-8 days, which affect the appearance of the raw prawns. However, the cooked product remained acceptable up to 12 days and on one occasion up to 16 days. Reilly et al. (1986) has observed that prawns lost their value as prime quality head-on produce after 2 days, during storage at $0^{\circ}C$. Reilly et al., (1985) found that prawns iced immediately kept for 17 days, while 4 h, 8 h and 12 h delayed iced prawns kept for 16, 13 and 11 days respectively.

The prawns used in this study were un-iced up to the time of landing, which is about 8-10 h, after they are caught. Though the prawns were acceptable up to 12 days on the "cooked-quality" score, they were rejected on "raw appearance" from the 10th day in ice.

During this study the pH of prawns increased from 7.31 on the 1st day to 7.94 on the 17th day. On the 12th day, at rejection, the pH was 7.86 (Figure 2). Many workers have found that pH has a

close relationship with organoleptic properties and was useful as an index of quality (Farooqui et al., 1978, Novak, 1973, Flores and Crawford, 1973 and Foda et al., 1984). A pH value of 7.7 is suggested as acceptable and 7.7 as poor quality prawns (Farooqui et al., 1978). Foda et al. (1984) also found a pH of 7.95-8.35 at spoilage. Novak (1973) has reported that at pH 7.7 the characteristic sweet flavour of fresh shrimp disappears completely and pH 7.9 denotes the onset of spoilage. At a pH of 7.9 the prawns were found to be completely spoilt.

The results in pH of this study shows similar trends with the pH rising from 7.31 to 7.75 on the 7th day in ice, at which point the prawns were classed as Grade II. The coefficient of correlation between taste panel scores and pH was -0.819 (Table 2), which is significant.

The total volatile nitrogen (TVN) and trimethylamine (TMA) contents are shown in Figure 3. There was a drop in the TVB level during the first 7 days of ice storage. From the 7th day onwards the TVB levels rose sharply from 27.7 mgN/100 g to 49.3/100 g on the 14th day in ice.

Reports on the value of TVB as an index of prawn quality is conflicting. Reilly et al. (1985) stated that TVN/TMA are not reliable as indices of quality. Boee et al. (1982) working on the storage of shrimp (Pandalus borealis) has observed that TVN increased evenly. Matches (1982) working on P. borealis stored at 5 different temperatures, found that TVN increased both with increase in time and temperature. Velankar et al. (1961) and Cann (1974) have found the increase in TVN to be low during the initial period of storage, with a rapid increase noted afterwards. Montgomery et al. (1970) and Cantoni et al. (1978), have suggested 30 mg/100 g N TVN as the limit indicative of fresh shrimp.

The results from the present study conforms to the level of 30 mg/100 g N for fresh prawns as suggested by Montgomery et al. (1970). After the 7th day in ice, the prawns showed signs of spoilage and at rejection on the 12th day, TVN value was 44 mg/100 g N, and on the 17th day when the prawns were completely spoilt it was 50.3 mg/100 g N. The coefficient of correlation between taste panel scores and TVN values was -0.941 (Table 2) indicating a high level of significance.

The TMA values increased gradually during storage, from an initial level of 1.7 mg/100 g N to 5.08 mg/100 g N on the 17th day. The correlation bewteen taste panel score and TMA was -0.984 which is very significant (Table 2). Initially the TMA level was very low, and though it increases steadily with storage, the level was too low to be of much value as an index of quality. Montgomery et al. (1970) has reported a level of 5mg/100g TMA as the limit to assess spoilage in Australia. During the present experiment, even at the time of rejection on the 12th day, the TMA level was only 4.5 mg/100 g N and it reached the 5.0 mg/100 g level only on the 17th day, at which point the prawns were completely spoilt. Reilly et al. (1985) has observed that the level of 5 mg/100 g was never reached during ice storage of prawns.

Cobb et al. (1973) has suggested that the adjustment of TVN values on the basis of amino acid content might improve the reliability of TVN data. They noted a ratio of 1.0 mg N/mM for fresh shrimp, 1.2 mg N/mM for shrimps of questionable acceptability and 1.5 mg N/mM for spoilt shrimps. The TVN/AA-N ratio obtained from this study (Table 3), were 1.2 mg N/mM from the 1st day, but remained less than 1.5 mg N/mM up to the 14th day. It was only on the 17th day when the fish was in an advanced state of spoilage that the TVN/AA-N ratio was 1.5 mg N/mM level as suggested by Cobb et al. (1973).

Indole is used as a measure of quality of prawns imported into the USA, by the US Food and Drug Administration (FDA). The FDA has set a limit of 25 mg/100 g, above which level prawns are considered spoilt. Chang et al. (1983) working on the effect of fresh storage temperature, freezing and boiling on the indole levels in shrimp found that at low temperature storage 0°C and 4°C) the indole levels, even in severely decomposed shrimps were lower than 25 mg/100 g. At higher storage temperatures (12°C and 22°C) very high indole levels had been reached, and therefore Chang concludes that indole levels indicate decomposition, although decomposed shrimp may not always contain indole. Matches (1982) working on storage of shrimp (P. borealis) at 0°C, 11.1°C, 16.7°C and 22.2°C reports that indole increased both with increasing time and temperature during storage. Boee et al. (1982) has found that indole level was low and constant initially in shrimp (P. borealis) stored at 0°C and 5°C for 20 days. The level had increased only as shrimp became putrid.

The bacterial count on the 1st day in ice was 3.0×10^5 and decreased slightly during the first week. After the 10th day in ice, the bacterial count increased rapidly to 1.8×10^6/g on the 14th day and 3.0×10^7/g on the 17th day in ice (Figure 5). On the 14th day the prawns were judged to be unacceptable by the taste panel (Figure 1). The coefficient of correlation between the taste panel scores and the total bacterial count was -0.869 and was significant. The counts indicate onset of spoilage after the 10th day, rather than the level of freshness. Velankar et al. (1985) also observed a decrease in the bacterial count during the initial days of storage. Surendran et al. (1985) observed an initial decline and attributes it to the death of cold sensitive species of bacteria.

The initial bacterial counts of tropical shrimp in ice, as reported by Cann (1977) is slightly lower (10^4/g range) but they have risen to 10^6-10^8/g on the 12th day in ice. The change in the count has not been significant till after the 6th day, after which it increased and this trend is similar to the results obtained during this experiment.

The succession of bacterial genera during the storage in ice is presented in Table 4. The initial flora on the prawns consisted of Micrococcus (26%), Staphylococcus 19%), Aeromonas (27%), Pseudomonas (19%) and Enterobacteriaceae was not isolated after the 1st day of sampling. On the 7th day of storage, Aeromonas was the predominant species (59%) and Moraxella (15%) which was not found in the first instance, was isolated. Only Micrococcus, Aeromonas and Moraxella were found on the 14th and 17th day in ice, after the samples were rejected by the taste panel. The predominant flora on the spoilt prawns were Aeromonas (40%) and Moraxella (40%).

There is a significant change in the flora during storage in ice. Initially a mixed flora of gram positive (44%) and gram negative (42%) bacteria were detected, while on the 7th day the gram positives comprised only 26%, while 69% were gram negative bacteria. At spoilage, 80% of the flora were gram negative. Of the six genera isolated initially, Pseudomonas, Enterobacteriaceae and Staphylococcus were not isolated after the first week.

Surendran et al. (1985) found Moraxella and Acinetobacter to be the predominant group at spoilage, in P. indicus, Metapenaeus dobsoni and M. affinis. Surendran and Gopakumar (1981) also reported that Moraxella and Acinetobacter comprised 74% of the spoilage flora in prawns. Reilly et al. (1986) working on the spoilage of P. mondon at different temperatures, found the initial microflora to be mesophilic and dominated by Enterobacteriaceae (20%), Aeromonas hydrophila (20%), Achromobacter sp. (20%) and to a lesser extent by Acinetobacter (13%), Pseudomonas (13.3%), Chromobacterium (6.7%) and Vibrio cholera (6.7%). At all storage temperatures Aeromonas hydrophila was found to be the principal spoilage organism. A. hydrophila which occurs naturally in fresh and brackish water, has been reported as a human pathogen causing travellers diarrhoea (Reilly et al. 1986).

Studies carried out on the flora of tropical shrimp (Cann, 1977) have shown that gram positives predominate initially, with over 50% comprising Coryneforms and Micrococcus sp.. Cytophaga Flavobacteria group had been present in significant numbers, and Achromobacter, Bacillus and Streptomyces had also been found. After spoilage in ice either Pseudomonas or Achromobacter (the present Acinetobacter - Moraxella group) predominated.

The results of this study also showed that gram positives predominate initially (44%), but 80% of the spoilage flora comprised of Aeromonas and Moraxella. The other 20% comprised of Micrococcus sp.

Smith et al. (1984) had found that Flavobacterium Aeromonas, and Yersinia, were able to produce indole in shrimp extracts. Aeromonas and Proteus exhibited proteolysis and were able to produce indole in the extract without added L-tryptophan. The indole level of prawn flesh increased steadily during the present experiment.

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Table 1

The change in raw appearance and grade of P. indicus during storage in ice

Day in ice	Grade	Raw appearance
1	I	Fresh characteristic odour; texture firm, colour normal
3	I	Fresh characteristic odour, texture firm, colour normal
7	II	Neutral odour; head loose; flesh (texture not very firm)
10	Just acceptable	Slight off odour, head very loose and black spots on head, texture of flesh soft
14	Reject	Strong off odour, heads very loose, most heads broken from body. Black spots on head and other parts. Texture of flesh soft.
17	Reject	Putrid odour. Black spots very severe. Texture of flesh very soft.

Table 2

Coefficient of correlation between overall taste panel score and other parameters measured

Parameters	Coefficient of correlation (r)
pH	-0.819
TMA	-0.984
TVN	-0.941
Indole	-0.951
Total bacterial count ($30^{o}C$)	-0.869

Table 3

The total volatile nitrogen (TVN) and amino nitrogen (AA-N) levels in P. indicus stored in ice

Day in ice	TVN mg N/100g	AA-N mmM/100g	TVN/AA-N ratio mg N/mM
1	28.1	10.8	1.35
3	24.8	18.5	1.33
7	27.7	22.3	1.25
10	37.9	28.8	1.32
14	49.3	36.3	1.36
17	50.3	23.4	2.15

Table 4

Succession of microflora during ice storage of prawns (P. indicus)

	Days in ice (% occurrence)			
	1	7	14	17
Micrococcus	26.0	11.0	32.0	20.0
Staphylococcus	19.0	15.0	-	-
Aeromonas	27.0	59.0	42.0	40.0
Moraxella	-	10.0	20.0	40.0
Pseudomonas	19.0	-	-	-
Enterobacteriaceae	6.0	-	-	-

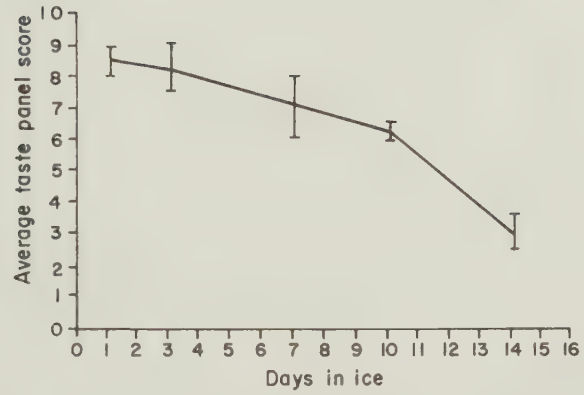

Figure 1 The taste panel evaluation of P. indicus stored in ice

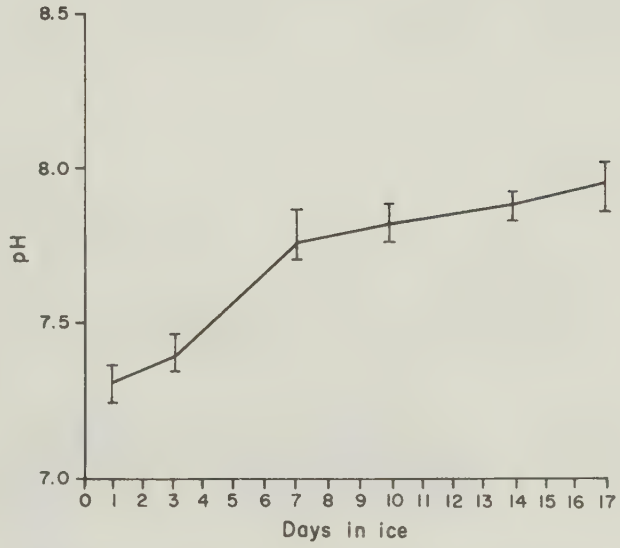

Figure 2 The pH of P. indicus stored in ice

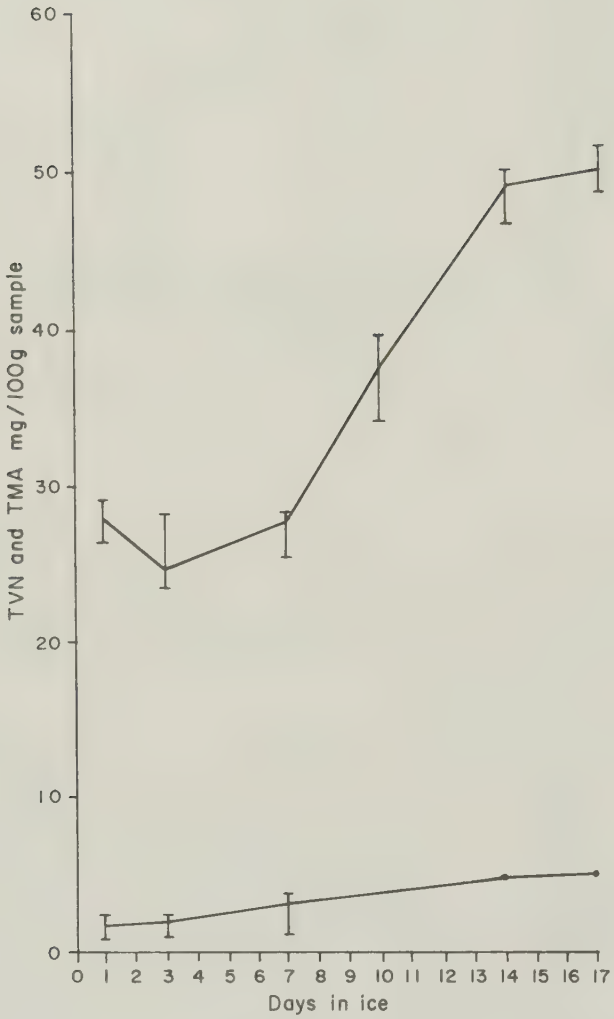

Figure 3 The total volatile nitregen (TVN) and trimethylamine (TMA) levels in P. indicus during ice storage

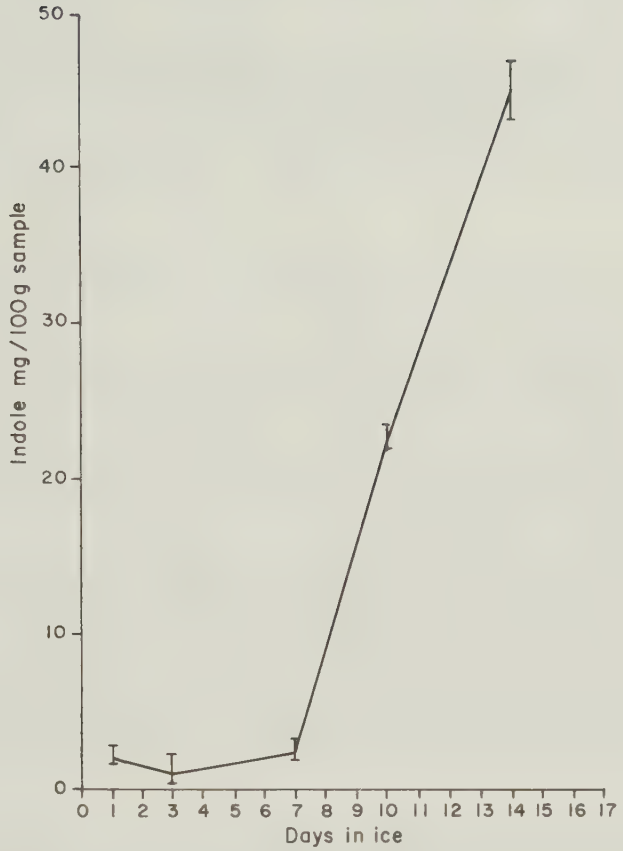

Figure 4 The indole level of P. indicus during ice storage

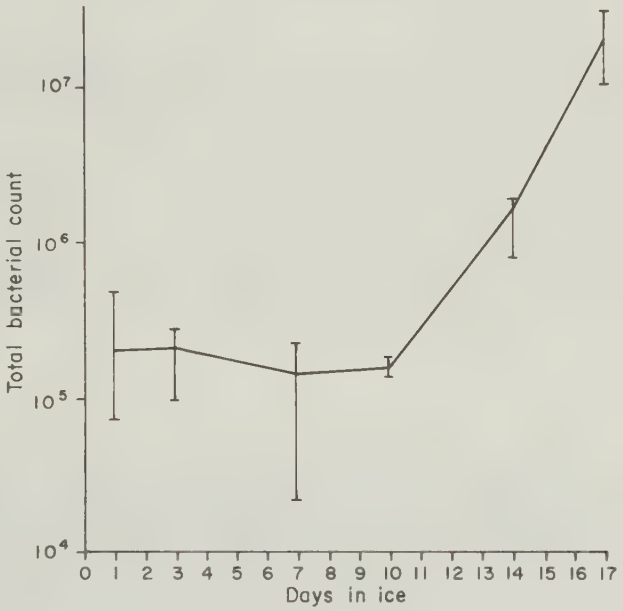

Figure 5 The total bacterial counts of P. indicus during ice storage

A STUDY OF AEROBIC MICROFLORA OF SHRIMP (PENAEUS INDICUS) CAUGHT IN THE SEA OFF NEGOMBO

by

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ABSTRACT

Prawn samples were collected from five boats at landing points in Negombo on the West coast of Sri Lanka over a period of six months starting from July 1984. The bacterial load and the qualitative microflora of the samples were investigated.

The samples tested showed total bacterial counts ranging from 1.9×10^6/g to 6.5×10^6/g at an incubation temperature of 30°C. At 5°C incubation the counts ranged from 1.9×10^5/g to 1.92×10^6/g.

Majority of organisms isolated at 30°C incubation were gram positive with Micrococcus (40.7%) predominating and with Bacillus showing a much lower incidence. Pseudomonas, Vibrio and Escherichia were in very low numbers with Vibrio parahaemolyticus and Vibrio alginolyticus being identified.

Over 70% of the isolates at 5°C were Micrococcus. The incidence of Vibrio and Bacillus species were extremely low at this incubation temperature.

1. INTRODUCTION

Frozen shrimp is the most important seafood product exported by Sri Lanka. Annual export of frozen shrimp earns considerable foreign exchange for the country. The quality changes and spoilage of shrimp is reported to be influenced by post-mortem nucleotide decomposition and other biochemical reactions (Stone, 1971; Flick and Lovell, 1972; Hashimoto, 1965; and Carroll et al., 1968). The role played by microorganisms in the spoilage of shellfish has been studied by many workers (Cann, 1977; Chai et al., 1965 and Herbet et al., 1971).

The quality of processed shrimp produced for the international market should conform to world standards. Absence of heavy bacterial loads, absence of pathogens and organisms of public health significance in the finished product, are the key factors governing the quality of this product. A prerequisite to the quality control and quality assurance of such a perishable product as shrimp is a study of microorganisms associated with the raw material and the role these organisms play in the spoilage of the product in our own environment. Some quantitative studies were carried out on the microflora of prawns (Sumner et al., 1979) in Sri Lanka. Data on the qualitative microflora of shellfish is non-existant for Sri Lankan prawns.

This study was planned to obtain information on the microbiology of shrimps which may be useful in the future development of the shellfish trade in Sri Lanka.

2. MATERIALS AND METHODS

2.1 Selection of Shrimp

Shrimp caught in waters off the sea coast of Negombo were collected from five landing points. The shrimp selected for this study belong to the species Penaeus indicus. Approximately 250 g of shrimp were collected in sterile polythene bags at each landing point. The bags were sealed with a rubber band and kept in an insulated box with flake ice, care was taken that no ice or ice water got inside the polythene bags. The samples were taken to the laboratory for analysis within 1 h.

2.2 Bacteriological Examination

2.2.1 Total bacterial counts

25 g of shrimp were weighed taking approximately 5 g from each of the 5 bags. The samples were weighed into sterile stomacher bags (Seward Laboratory Stomacher) and 225 mL of sterile peptone water added and stomached for 30 s. The standard sample homogenate was similarly diluted using peptone water as the diluent. The suitable dilutions were plated onto nutrient agar (Difco).

Both standard pour plate and spread plate techniques were employed. The plates were dried and incubated aerobically at 30°C and 5°C. After 48 h of incubation at 30°C and 7-10 days incubation at 5°C, total bacterial counts were determined.

2.2.2 Isolation, characterisation and identification of bacteria

Five representatives from each of the dominant types of colonies were picked off from the selected nutrient agar spread plates. The selected colonies were purified by restreaking and transferred to stock culture bottle and stored at 5°C.

The colony morphology, pigmentation, motility, staining characteristic and capsules were observed after recovery of isolates from the stock cultures.

The other biochemical test performed for characterisation included oxidase, catalase, oxidation and fermentation of glucose in Hugh and Leifsons medium, fermentation and production of acid from glucose, production of acetyl methyl carbonyl from glucose, phosphate broth, mixed acid fermentation and tryptone broth for indole production. The generic classification of bacterial isolates were carried out using the scheme of Shewan et al., (1960), Cowan (1977), and Lee and Pfeifer (1975).

The identification of Gram positive coccoid bacteria was carried out according to Baird-Parker (1966). Gram positive endospore forming rods were identified according to Gibbs and Shapton (1968) and Cowan (1977). Identification of Gram negative rods were carried out according to scheme of Hendrie, Mitchell and Shewan (1968) and Collins and Lyne (1979).

3. RESULTS

The total bacterial counts at incubation temperatures 30°C and 5°C are in Table I. The total bacterial count of prawns at 30°C ranged from 1.90×10^6/g to 6.5×10^6/g with the average bacterial count of 3.93×10^6/g. The total count at 5°C ranged from 1.9×10^5 to 1.92×10^6/g with the average count 1.06×10^6/g.

Table 2 shows the numbers and the percentages of the different groups of Gram positive and Gram negative bacteria isolated from 30°C and 5°C incubated plates. About 42% of the isolates from 30°C incubated plates were found to be Gram positive coccoids which increased to 72% in the 5°C isolates. The Gram negative rods constituted over 35% of the isolates at 30°C which decreased to 22% for 5°C incubation.

Table 3 gives the percentages of different bacterial genera isolated from shrimp at the two incubation temperatures.

Table 4 illustrates the organisms identified at two different incubation temperatures with their respective percentages. Minor variations existed in the microflora of shrimp detected at two different incubation temperatures. However, the dominance of Micrococcus spp were quite clear. At 30°C incubation, a higher percentage of Vibrio spp and Bacillus spp were noted compared to 5°C incubation.

4. DISCUSSION

The total bacterial counts of the shrimp ranged from 1.9×10^6/g to 6.5×10^6/g (Table 1) at 30°C incubation. According to Cann (1977), Malaysian shrimp recorded counts in the same range and the counts for prawns from Thailand ranged from 10^3-10^6/g. Further the results were also consistent with the counts recorded by many other workers in the tropics (Venkataraman et al., 1953; Pillai and Nayar, 1962 and Jacob et al., 1962). However the counts reported by Sumner et al. (1982) during the survey of prawn processing factories exceeded 10^7/g for more than 50% of the samples analysed. The prawns in the factories are received from the eastern and northern part of Sri Lanka and a number of factors such as delay in transportation, insufficient icing and dirty containers contributed to bacterial build up.

Shrimp incubated at 30°C had bacterial counts slightly higher (Table 1) than the counts for shrimp incubated at 5°C. This can be attributed to dominance of mesophilic bacteria over the psychrophilic bacteria in the warm waters surrounding Sri Lanka. The counts at higher incubation temperature gives a better understanding of the microbial load of the shrimp. Similar variations in the total bacterial counts were observed by Poulter et al. (1981) and Shewan (1977) in tropical fish.

Gram negative Achromobacter and Pseudomonas are the dominant bacterial genera in temperate shrimp whereas in tropical shrimp gram positive Micrococci and coryneform groups appear to be the common microflora (Cann, 1977). In this study at 30°C incubation a high percentage (64%) of gram positive bacteria consisting of Micrococci and Bacillus were noted.

However it should be emphasised that in this study coryneform bacteria were not present in any of the samples in contrast to the findings of Cann (1977) and Sreenivasan (1959). Chinivasagam (IPHT-NARA, personal communication) working on fish (trenched sardines) caught from the same water as the shrimp, did not detect coryneform bacteria.

Micrococcus, Bacillus and Vibrio were the most common bacterial genera isolated from shrimp at 30°C incubation. Pseudomonas, Aeromonas and Enterobacteriaceae were isolated to a much lesser extent (Table 3). At 5°C incubation, organisms of the same genera were isolated but the incidence of Vibrio and Bacillus were reduced to a very insignificant level. Vibrio were identified as V. anguillarum, V. alginolyticus and V. parahaemolyticus (Table 4). V. parahaemolyticus is known to grow between 10°-44°C with an optimum temperature of 35°-37°C (Horie et al., 1966) and is sensitive to freezing and cold storage (Covert and Woodburn, 1972; Thomson and Thacker, 1973 and Johnson and Lister, 1973). V. alginolyticus is reported to respond more sharply to higher ambient temperatures than V. parahaemolyticus (Barrow and Liston, 1970) and it was isolated more frequently from coastal sea water and from shellfish during warmer months of the year (Qadri and Zuberi, 1971 and Sakazaki, 1965). The reduction of Vibrio in this study may be due to low selectivity of these organisms at 5°C incubation. A similar reduction of some mesophilic species probably would have caused the increase in Micrococcus at low incubation temperature. Micrococcus luteus was most common at both temperatures (Table 4). V. parahaemolyticus and to a lesser extent V. alginolyticus are considered food-borne pathogens, whereas V. anguillarum is non-pathogenic (Baross and Liston, 1970). The organisms of public health significance isolated in this study were only V. parahaemolyticus and E. coli. The percentage of E. coli isolated was very low and indicate that the faecal contamination of the environment as a possible source of contamination of the shrimp. No Salmonella was detected during this survey.

Pseudomonas, Achromobacter and Acinetobacter species are considered to be active spoilage bacteria in fishery products and are not numerous in tropical products (Shewan and Hobb, 1967 and Castell, 1973). In this study low percentages of Psuedomonas and Achromobacter were recorded. A similar low percentage of Pseudomonas and Achromobacter was recorded by Cann (1977) in shrimp from Thailand.

Like shrimp from most tropical environments, the shrimp in this study had a fairly high bacterial load. However the organisms of public health significance, food pathogens and active bacterial spoilers were found at very low levels in this study.

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Table 1

The total viable bacterial counts of shrimp (Penaeus indicus) in relation to incubation temperatures

Sample	Total bacterial count/g at 5°C	Total bacterial count/g at 30°C
A	1.9×10^5	1.9×10^6
B	9.5×10^5	4.2×10^6
C	3.3×10^5	6.5×10^6
D	1.83×10^6	3.3×10^6
E	1.9×10^6	2.73×10^6
F	9.6×10^5	4.95×10^6
Average count	1.06×10^6/g at 5°C	3.93×10^6/g at 30°C

A, B, C, D, E and F were six samples of shrimp examined in this study

Table 2

Numbers and the percentages of gram positive cocci, gram positive sporogenous rods, gram positive asporogenous rods and gram negative rods isolated at 30°C and 5°C from shrimp (Penaeus indicus)

Type	Number of isolates at incubation temp. 30°C	Number of isolates at incubation temp. 5°C	Percentage at 30°C	Percentage at 5°C
Gram positive cocci	12	13	42.8%	72.22%
Gram positive sporogenous rods	6	1	21.4%	5.55%
Gram positive sporogenous rods -	-	-	-	-
Gram negative rods	10	4	35.71%	22.22%
Total	28	18	-	-

Table 3

Numbers and percentages of different bacterial genera isolated from shrimp (Penaeus indicus) at 30°C and 5°C

	Number of isolates at incubation temp. 30°C	Number of isolates at incubation temp. 5°C	Percentage at 30°C	Percentage at 5°C
Micrococcus species	11	13	40.74%	72.21%
Staphylococci species	1	-	-	-
Pseudomonas species	3	1	11.1%	5.5%
Bacillus species	6	1	22.2%	5.5%
Vibrio species	4	1	14.8%	5.5%
Aeromonas species	1	1	3.7%	5.5%
Entero-bacteriaceae species	1	1	3.7%	5.5%

Table 4

Identified bacteria with the percentage incidence in relation to incubation temperature

Organisms	Number of isolates at 30°C	Number of isolates at 5°C	Percentage (30°C)	Percentage (5°C)
Staphylococcus epidermidis	1	-	3.57	-
Micrococcus luteus	6	11	21.4	61.1
Micrococcus roseus	1	-	3.57	-
Micrococcus species (?)	4	2	14.1	11.1
Vibrio parahaemolyticus	1	-	3.57	-
Vibrio anguillarium	-	1	-	5.55
Vibrio Alginolyticus	3	-	10.7	-
Escherichia coli	1	1	3.57	5.55
Aeromonas species (?)	1	1	3.57	5.55
Achromobacter Acinetobacter	1	-	3.57	-
Bacillus species (?)	-	1	-	5.55
Bacillus circulans	1	-	3.57	-
Bacillus alvei	1	-	3.57	-
Bacillus coagulans	2	-	7.14	-
Bacillus coagulans	2	-	7.14	-
Bacillus licheriformis	2	-	7.14	-
Pseudomonas cepecia	1	1	3.57	5.55
Pseudomonas fragi	1	-	3.57	-

MICROBIOLOGY OF PRAWN PROCESSING

by

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ABSTRACT

Microbiological quality indices, namely standard plate count (SPC), presence/count of V. parahaemolyticus, V. cholera, S. aureus, Salmonella sp and faecal coliforms, were determined at specific points in the preparation of frozen prawns (Penaeus monodon). Plant workers' hands were examined for the presence of S. aureus.

Seven prawn farms and 10 frozen prawn processing plants, the majority of which are in Luzon, were surveyed. Changes in the SPC were detected during the process but the final products had SPC within USA and Japanese market standards. V. parahaemolyticus was present in newly-harvested prawns. The level of faecal coliforms varied depending on pond and plant. V. cholera was detected twice throughout the pond and plant survey, while Salmonella was absent in all samples taken for analysis. About 50% of plan workers showed the presence of S. aureus.

A survey of prawns sold in 4 major markets in the Metro Manila area showed the absence of Salmonella in the gut and flesh; V. cholera was detected in 12 out of the 128 samples analysed.

INTRODUCTION

The need for a more effective seafood quality control and inspection programme has been repeatedly brought up in national and international consultations. The 1987 consultation sponsored by FAO in India presented data on rejection of seafood exports from Asian countries for reasons that vary from simple mis-labelling to spoilage (INFOFISH, 1987). The rejections represented not only monetary loss to the exporter but more significantly, injury to the industry.

Rejection of products at the importing port denotes non-conformity with product standards. Microbiological standards are largely based on the observance of good manufacturing practices and proper personnel hygiene. It is assumed that the products have a baseline count that is kept under control during processing. In the case of prawns however, the baseline microbiological parameters have been derived from those prawns occuring under temperate conditions. There appears to be evidence that the flora as well as the load of temperate prawns differ from those of tropical prawns (Cann, 1977); thus, standards for tropical prawns formulated on data from temperate countries may not be rational. At present however, data are insufficient to support a revision of standards. This study if part of a regional effort to provide data on prawn and prawn processing that reflect the existing situation in tropical countries. Changes in microbiological quality with emphasis on microorganisms of public health significance were followed from harvest to the processing of the frozen product. Brackishwater farmed prawns, Penaeus monodon were used. A market survey to determine the incidence of Salmonella sp. and V. cholera was also undertaken.

MATERIALS AND METHODS

Sampling and Examination of Frozen Prawns

Representative samples weighing 250 g (5-9 pcs) were withdrawn at specific points during harvest and processing and were assessed microbiologically.

Sampling points: The following stages of the process were considered critical and were designated as sampling stations.

1. At pond site:
   a. after harvesting
   b. after washing

2. In the processing plant:
   a. receiving
   b. sizing/sorting
   c. after washing
   d. layering
   e. frozen product

Prawns were placed in sterile stomacher bags which were kept in ice during transport to the laboratory for examination.

Microbiological Examination

Prawns were examined for the following:

a. Standard plate count
b. Vibrio parahaemolyticus count
c. Vibrio cholera
d. Salmonella sp.
e. Faecal coliform count
f. Staphylococcus aureus count

Sample preparation and analysis followed the procedures of ICMSF (1974, 1986). Approximately 250 g of prawns were cut into small pieces, and mixed to form a composite sample. A 30 g portion was mixed with 270 mL of 0.1% peptone water and blended in a stomacher. The resulting homogenate was used for all microbiological tests except for detection of Salmonella.

Standard Plate Count (SPC)

Standard Plate Count determinations were carried out by spreading diluted aliquots (0.1 mL) on iron agar (Jensen and Schultz, 1980). Duplicate plates were incubated at 37°C for 24-48 h.

Vibrio parahaemolyticus

Vibrio parahaemolyticus counts were obtained following incubation at 37°C for 24-48 h of duplicate plates of Thiosulfate Citrate Bile Sucrose (TCBS) agar surface inoculated with 0.1 mL of suitable dilutions of the sample homogenate. Colonies with typical appearance of V. parahaemolyticus (bluish-green colonies, sometimes colorless with green centre, 2-4 mm in diameter) were counted.

Vibrio cholera

V. cholera was detected by inoculating 0.1 mL homogenate on the surface of TCBS agar and incubating at 27°C for 24-48 h. Presumptive or tyical colonies; (large, yellow, slightly flattened with opaque centres and translucent peripheries) were picked out and tested for catalase and oxidase reactions. Colonies which are both catalase-positive and oxidase-positive were subjected to confirmatory test using API 20E (API International Geneva, Switzerland).

Salmonella sp.

The presence of Salmonella was detected by homogenizing a 25 g portion of the composite sample in 225 mL (pH 7.2) sterile buffered peptone water and incubating for 24-48 h at 37°C. A 1 mL sample was transferred to duplicate tubes of Tetrathionate and Selenite Broths (9mL), incubated for 24 h at 37°C and sub-cultured into Xylose Lysine Desoxycholate (XLDA) and Bismuth Dulphite Agars (BSA) (Oxoid Manual, 1982). After incubation for 24 h at 37°C, suspect colonies were streaked into Nutrient Agar and later tested for catalase reaction and gram reaction (Salmonella: catalase +; gram -). Confirmation was done using API 20E.

Faecal coliforms (E. coli)

Feacal coliforms were enumerated using the 3-tube Most Probable Number technique. One mL of the diluted sample was inoculated into tubes of Brilliant Green Bile Broth (BGBB) and incubated at 44°±0.1°C. Tubes showing gas after 24-48 h were considered positive for faecal coliforms. The MPN was determined by reference to MPN Tables.

Staphylococcus aureus

S. aureus counts were carried out by spreading diluted aliquots (0.1 mL) on duplicate plates of Baird-Parker (BP) agar and incubating at 37°C for 24-48 h. Typical colonies were counted.

Assessment of Personnel Hygiene

Plant workers were examined for the presence of S. aureus on their hands. The workers were asked to place the tip of their fingers (with or without gloves) on to plates of Baird-Parker Agar which were subsequently incubated at 37°C for 24-48 h and examined for typical S. aureus colonies. In total 229 workers from 10 processing plants were so tested.

Sampling and Examination of Market Prawns

A survey was conducted to determine the incidence of Salmonella sp. and V. cholera in the gut of prawns sold in wet markets. Four wet markets were included in the survey and in each market, samples were obtained from as many stalls as there are different sources. Sampling was done over a period of 7 months and a total of 128 samples coming from 15 sources were tested. Each sample lot consisted of 5-9 pieces weighing approximately 250 g.

Sample preparation

Gut was removed and mixed with inner flesh; the gut-flesh mixture was weighed into 30 g and 25 g portions. The 30 g portion was used for detection of V. cholera and screening for Salmonella. The screening consisted of testing the sample homogenate for Enterobacteriaceae; only those which yield Enterobacteriaceae count of 10^5/g or higher are selected for Salmonella testing (Sumner, 1980). The 25 g portion was kept in the refrigerator and used to test for Salmonella if the screening test so warranted. The maximum time between sampling and testing for Salmonella was 48 h.

RESULTS AND DISCUSSION

Harvest Site and Conditions

Of the 7 ponds surveyed, 6 are located in Luzon, 200-300 km from Manila and one in the Visayas, about 5 km from the nearest city (Figure 1). Pond 1 is in the intensive culture scale, the others are semi-intensive. The prawns were 3-4 months old at harvest time.

Harvesting, which may take 8-15 h, was done by drainage of the pond water into bagnets and unloading of the prawns into water tanks. The tanks contained water whose temperature varied from at -0.8°C to 0.14°C and chlorine level ranged 10-30 ppm. In one pond (Pond 1) the wash water was not chlorinated. Only in Pond 4 was temperature of the washing water monitored. Wash water was negative for coliforms. Pond 3 used a 2-step procedure in washing the prawns.

Grading for size and soft-shelled price was done at the pond site. Transport was by non-refrigerated vans, either direct to the processing plant or to the fish landing area. The prawns were iced, (1:2, 2:1 or 1:1; ice: prawns) prior to packing in styropore boxes which may or may not be lined with plastic. In pond 4, metal boxes were used with styropore insulation. Re-icing during transport was not practiced.

Processing Plants

Plants A to D correspond to Ponds 1-4. Plants B, C and D are outside Metro Manila; all others were located in Metro Manila. The production rate of plants range from 3-6 t per day. The raw materials reach the plants by land, by sea or air. Transport time can be as short as 2 h or as long as 30 h (by boat). Plants in the Metro Manila area avail themselves of the water supply from the local waterworks system. Those outside Metro Manila have deep wells and the water is chlorated at levels close to 0.2 ppm.

Prawns are normally washed by repeated immersion of trays containing the prawns in chilled chlorinated water. Chlorine level in the water ranges from 10-50 ppm and successive dippings can be as many as 5 times, 3 times on the average. Water is changed when it becomes cloudy or in certain cases after 10 immersions. In some plants the initial washing is done in chilled chlorinated water and subsequent dips are in chilled water. One plant does not use chlorine in the wash water in accordance with requirements of the importing country. Only 2 of the 10 plants surveyed have quality control laboratories with facilities for microbiological work.

Footbaths were present except in 3 out of the 10 plants included in the survey. Where present, chlorine concentration in the footbath ranged from 150 to 400 ppm. One plant used a specific brand of liquid detergent instead of chlorine. Handwashing facilities are available in the ratio of 1 basin per 8 persons to as high as 1 per 45 people.

Some plants have a regular schedule for washing of hands and gloves, (e.g. every 15 min) while others require their workers to wash their hands everytime they leave and enter the processing area. Workers' outfits consisting of boots, head caps, face mask, gloves and aprons are provided. Comfort

rooms are available but in most of the plants there is only one comfort room each for male and female workers. In one plant, there are 3 comfort rooms for male workers and another 3 for female workers.

Personnel are briefed on plant and personal hygiene when they first start working. Few of the plants have regular seminars for this purpose but briefing is done on an individual basis. Two of the plants conduct regular health checks of their employees.

Standard Plate Count

The initial load of prawns of 4.5×10^3 to 1.0×10^5 cfu/g at 37°C (Figure 2) were comparable with SPC data obtained on similar species (Reilly et al., 1984). The values however are lower compared with the reported counts for temperate species, 10^4-10^7 at 20°C for shrimps from the Gulf of Mexico, 10^4-10^7 for the deep water shrimp Pandalus borealis and P. montagni (various authors cited by Cann, 1977). These counts were obtained at 20°C; counts at 37°C are often an order of magnitude lower.

Washing in the pond did not produce a definite reduction in SPC as had been expected. In more than 50% of the ponds surveyed, SPC actually increased after washing. Water chilled to a temperature of -0.8 to 0.14°C by the addition of ice was used for washing. The quality of water used for ice manufacture and the use of still baths for washing could possible account for the negative effect of washing. Harvesting is done by draining the pond and trapping the prawns into a bagnet. The first batches of prawns are cleaner because they go out with a large volume of pond water and are washed in fresh, chilled water. As harvesting progresses, the water gets dirtier and the prawns correspondingly dirtier. The situation is aggravated by the deteriorating condition of water in the still bath. In general, a supply of running potable water is not yet available in pond sites in the country; often, pond water is used for washing.

The effect of chlorine in the wash water was also not definite. While the increase in count in Ponds 1, 6 and 7 (Pond 6 and 7 not shown in Figure 2) could be attributed to unchlorinated water, a similar increase in Pond 3 could not be similarly explained. In the latter, the increased count could be attributed to the practice of not removing the prawns from the net prior to washing. Adhering dirt and contaminants especially in prawns at the middle of the batch might not have been completely washed off.

After transport, SPC increased in 3 out of 4 instances. Prawns were iced during transport but re-icing in general, is not practiced. Some plants upon receipt of the raw materials would wash them in chilled chlorinated water while others let the prawns await processing. In medium-scale plants, the prawns were processed immediately. Plant F put the prawns in the chiller while awaiting processing.

Sizing and sorting for soft-shelled pieces are carried out manually and the prawns are sometimes placed on the sorting table without ice.

The washing step after sorting and sizing yielded variable results as a function of chlorine in the water. Washing with chlorinated water decreased SPC but samples where unchlorinated water was used also showed a decrease (Plant H) or very little increase (Plants E and I). It also appears that the chlorine concentration was not strictly monitored. Water is used 3 to 10 times before it is replaced.

During panning, an increase in SPC was observed especially for whole prawns relative to the headless form.

Block frozen prawns gave an SPC of 1.7×10^3 - 1.7×10^4 cfu/g in the case of headless products and 6.1×10^3 - 9.3×10^5 cfu/g in the case of head-on prawns. Based on the SPC only, the products would have passed USA and Japanese standards.

Vibrio parahaemolyticus

This organism was always detected in newly harvested prawns where levels varied from 10^2 - 10^3 cfu/g (Figure 3). Washing at the pond site reduced the count slightly as did the other processing steps. The frozen products had presumptive V. parahaemolyticus counts ranging from nil to 10^2 cfu/g.

Faecal coliforms

Table 1 shows the faecal coliform count in freshly harvested and newly received prawns. Processing caused a significant reduction in the coliform load; it can also be observed that where the coliform count of the incoming product is high, i.e. more than 20/g, the faecal coliform count in the product after layering is still above the standard. Assuming that no significant change takes place during freezing, this would also imply that the finished products would not meet

standards. Plant G is an exception because a high coliform count at the layering section produced a product with a low count (less than 3/g).

When headless product is required, the increased handling during beheading raises the risk of contamination from workers' hands. In the present study, no increase in coliform count was observed, implying to some extent the absence of faecal contamination from workers' hands.

Staphylococcus aureus

S. aureus was detected at harvest in 5 out of 7 ponds surveyed. The numbers were low, ranging from 1.4×10^2 to 4.2×10^3 cfu/g, and did not exceed 10^4 in any plant (Figure 4). Sizing and sorting did not cause an increase in S. aureus counts, and neither did the increased handling during the production of headless frozen prawns.

Vibrio cholera

V. cholera was present in 2 of the 15 samples taken. It was detected in Pond 6 (not shown in Figure 2) at harvest and after washing. It was also found in the incoming raw material in plant A, but subsequent washing eliminated the organism. Reilly (1986) reports isolated of V. cholera in brackishwater prawn and suspected that the bottom mud is the source. Plant A had a common entrance for plant workers and for raw materials; the receiving area was also very open.

Salmonella

Salmonella was not detected throughout processing nor in any of the plants' incoming materials, similarly the organism was also absent in pond samples.

Plant Personnel Hygiene

The impact of personnel hygiene is greater in the case of frozen products than in the case of canned products which are handled minimally after the major sterilizing process. S. aureus is a communal inhabitant of skin, and it is estimated that more than 50% of healthy human adults carry the organism. Prawns are susceptible to contamination because of the amount of handling they undergo. Personnel hygiene becomes even more critical in the case of cooked prawns which are handled after cooking and normally do not receive further heat treatment before consumption.

The survey showed that S. aureus can be present in as many as 86% of the workers in the plant, with an average of close to 50% (Table 2). The situation would be alarming, were it not for the results of the examination on the products which showed very low S. aureus counts. The discrepancy can be partly traced to the washing in almost every step.

It is also shown that the use of gloves does not guarantee the absence of S. aureus (Table 3). While the incidence of S. aureus in workers wearing gloves is lower than in those who do not (19.4 vs 26.5%), individual results show that even with the use of gloves, the incidence of S. aureus can be as high as 53%. Disposable gloves are generally not used in the country's processing plants; more common are the PVC gloves which can be washed and re-used. Inefficient washing and sanitizing can make re-usable gloves a source of contamination rather than protection for the products. Still some workers could have the notion that gloves are mainly for their own protection rather than to minimise product contamination from their hands.

Market Survey

Of 100 samples obtained from 4 major markets over a period of 6 months and 28 samples from 3 markets obtained within a span of 1 month, Salmonella sp. was not detected but V. cholerae was detected in 12 out of the 128 samples examined. The origin of samples positive for V. cholerae can be narrowed to 5 out of the 14 sources for the samples bought from the various markets. The temperature of the prawns in the retail markets varied from 13.8°C to 35.2°C (Figure 5).

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Table 1. Faecal coliform count (MPN/g) of *P. monodon* at harvest and during processing

Source	Pond site		Processing step				
	Harvest	Washing	Receiving	Sizing/sorting	Washing	Layering	Frozen product
Pond 1 - Plant A	nil	nil	nil	nil	nil	nil	nil
Pond 2 - Plant B	nil	nil	nil	nil	nil	nil	nil
Pond 3 - Plant C	nil	nil	nil	nil	nil	nil	nil
Pond 4 - Plant D	-	-	-	-	-	-	-
Plant E			150	9	15	7	
Plant F			1100	90	23	23	
Plant G			1100	1100	28	120	23
Plant H, T_1			23	9	nil	nil	
T_2			-	nil	9	nil	
Plant I			90	40	70	15	
Plant J, T_1			240	43	23	9	
T_2			4	75	4	nil	

Table 2

Incidence of S. aureus in plant workers' hands

Company	Total work force	No. of workers tested	Incidence of S. aureus (% of workers tested)
A	120	30	66.7
B	100	16	25.0
C	75	29	17.2
D	190	27	55.0
E	70	15	86.7
F	196	20	0
G	180	30	20.7
H	90	16	25.0
I	50	16	50.0
J	110	30	66.7

Table 3

Use of gloves and incidence of S. aureus on plant workers' hands

	Gloves used		Gloves not used	
Company	No. of workers	% positive	No. of workers	% positive
A	9	33.3	21	81.0
B	16	25.0	0	0
C	29	17.2	0	0
D	0	0	0	0
E	2	0	13	100.0
F	20	0	0	0
G	26	15.4	4	50.0
H	1	0	15	80.0
I	13	53.8	3	33.3
J	2	50.0	28	67.8

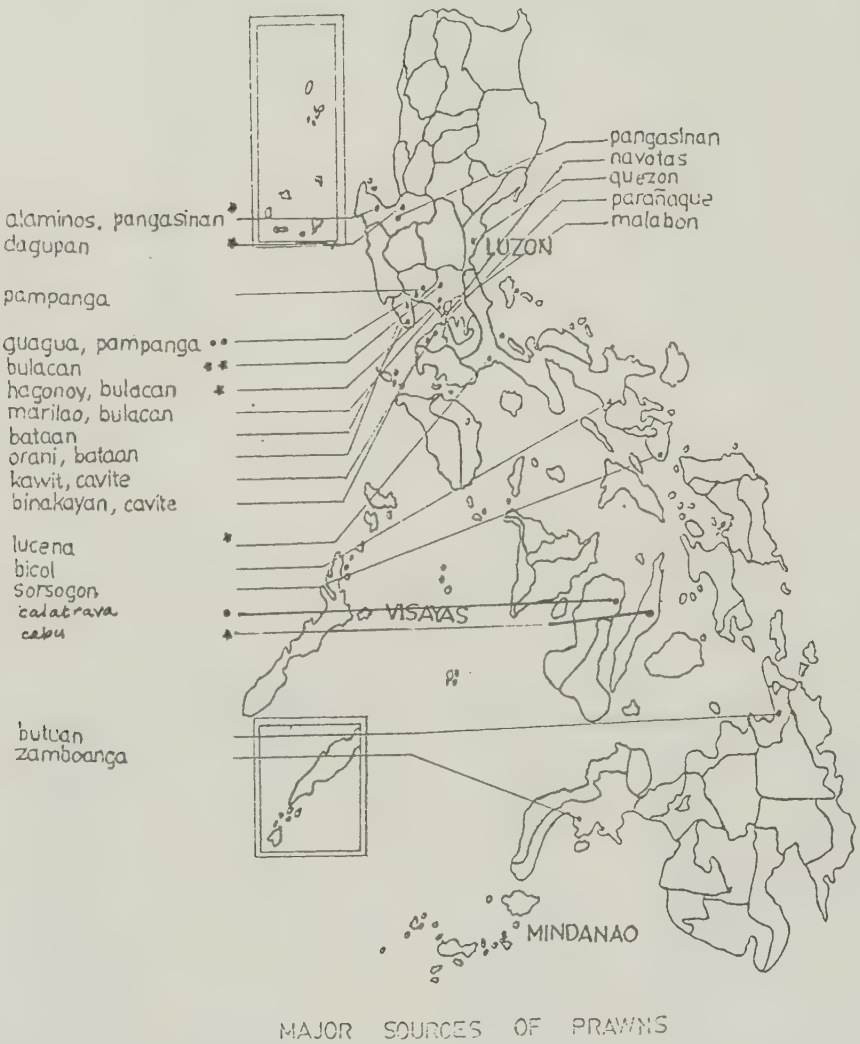

Fig. 1 Sampling sites for prawn farms (*) and processing plants (.)

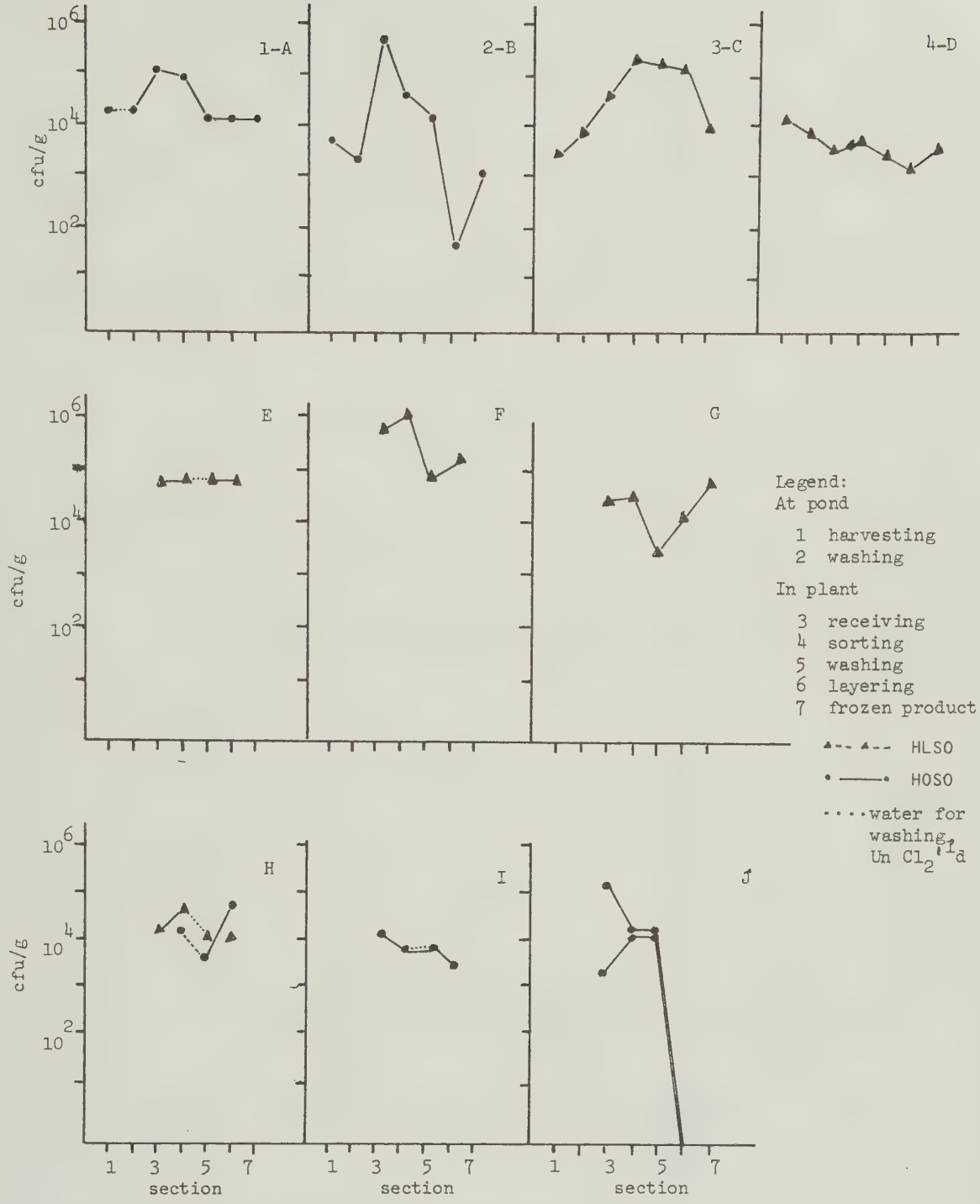

Fig. 2. Standard Plate Count (cfu/g) of P. monodon at various stages of processing

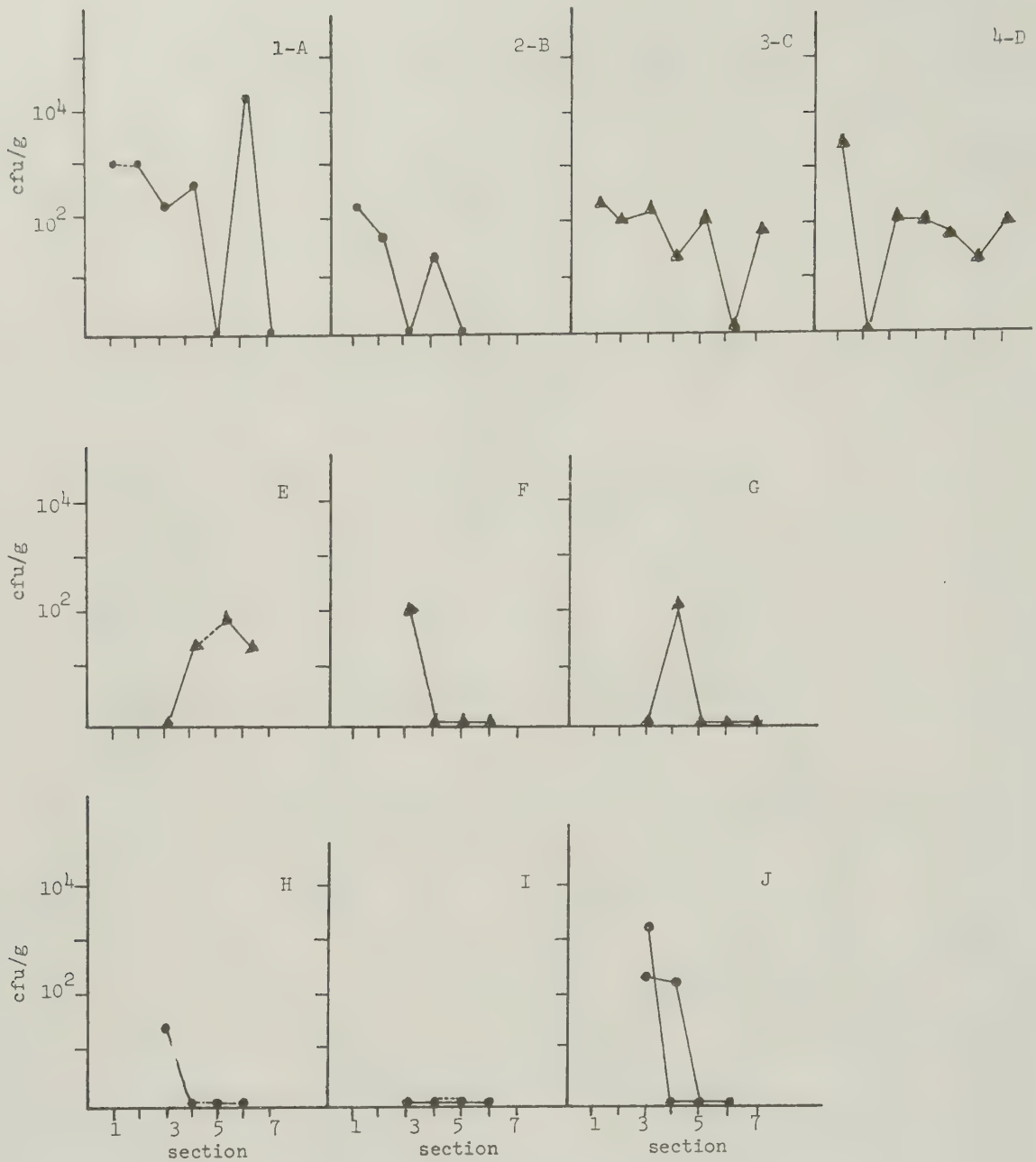

Fig. 3. V. parahaemolyticus in P. monodon at harvest and during processing.

HLSO

HOSO

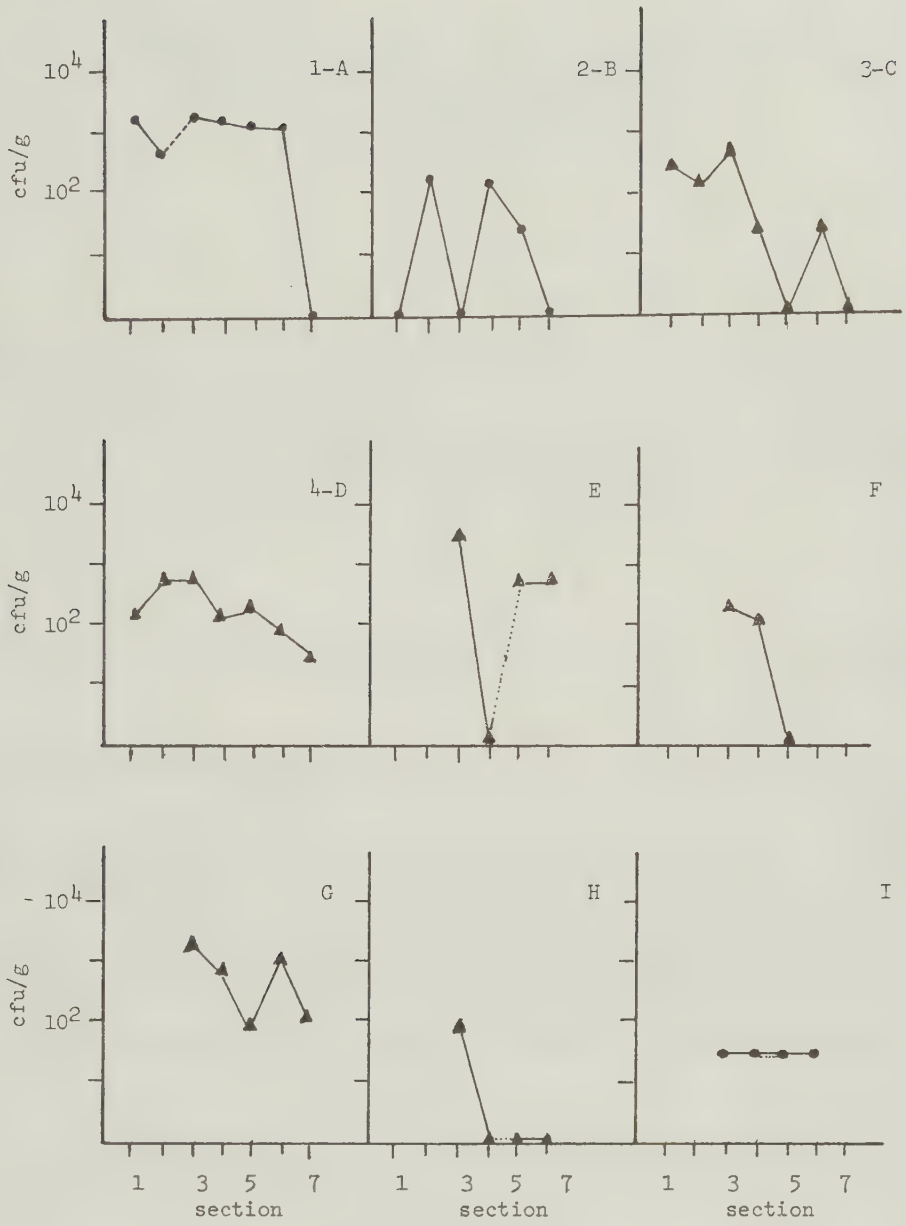

Fig. 4 . S. aureus count in P. monodon at harvest and during processing.

HLSO
HOSO

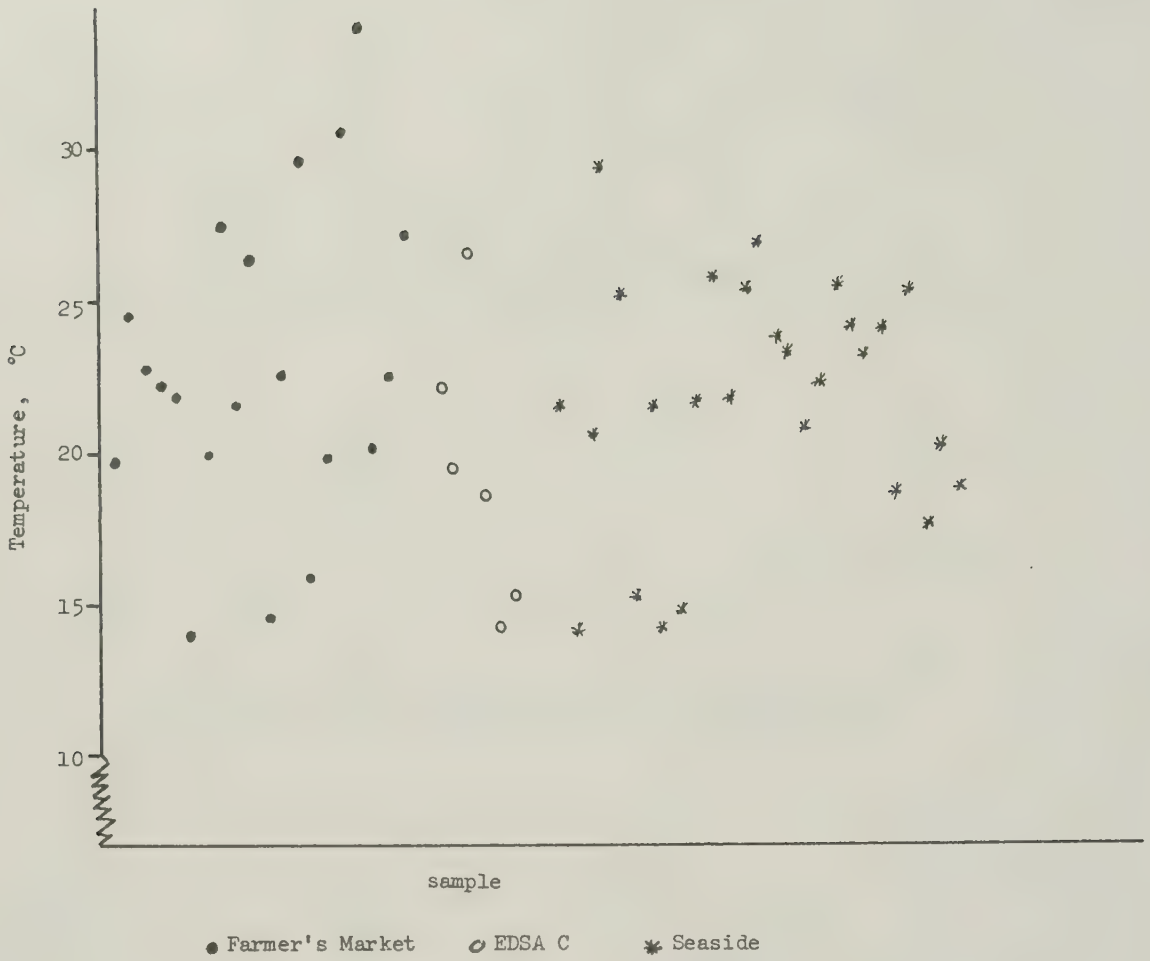

Fig.5 Temperature of prawns retailed in 3 markets in Metro Manila

REPORT ON PRAWN HYGIENE PROJECT

by

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ABSTRACT

Determination of Enterobacteriaceae and Salmonella in the gut and on the surface of Indonesian marine shrimp was carried out. The results of all samples (gut and surface) of the shrimp was negative for Enterobacteriaceae and Salmonella. The reliability of the analytical method was confirmed by positive results of Enterobacteriaceae and Salmonella for inoculated shrimp samples with Salmonella. This result probably indicates that Salmonella is not found in the marine shrimp naturally and any Salmonella in the marine shrimp is probably due to contamination during handling, transportation or processing.

INTRODUCTION

Indonesia produces and exports shrimp in significant amounts. In 1985 shrimp production in Indonesia was 110,177 t and in the same year, 30, 984 t of it was exported to Japan, Europe and America.

One of the problems associated with the development of shrimp production and export is microbial contamination especially Salmonella.

In order to get more information on the salmonella (as the enterobacteriaceae) contamination in shrimp products, the national centre for fishery quality control and processing development (Indonesia) and FAO worked together on a prawn hygiene project.

In this project, microbiological work for salmonella and enterobacteriaceae in the gut and on the surface of shrimp was carried out using standard methods recommended by FAO. Based on the data produced, it was possible to determine where the contamination occurred in order to reduce Salmonella contamination.

MATERIALS AND METHODS

Sampling

Samples of marine shrimp and gut were collected between March and December 1987; 30 samples of shrimp gut were collected from a frozen shrimp factory (PT. Pumar) in Jakarta and 30 samples of whole shrimp were collected from fishermen who usually catch shrimp on the north coast of West Java.

All of the samples were taken by laboratory staff using sterilized tongs and placed in sterilized polythene bottles, closed and placed in ice at approximately 0-1^{o}C for 2 hours before analysis for shrimp gut and 12 hours for whole shrimp.

The sample was analysed microbiologically by the following scheme:

Microbiological Examination

Enterobacteriaceae and Salmonella determinations were done using the procedure as developed at the University of New South Wales (Department of Food Science and Technology) as below:

Enterobacteriaceae determination

a) Shrimp gut

Shrimp gut was weighed aseptically (25 g) into a sterile blending container. To this 225 mL peptone solution (0.1%) was added and blended at high speed for two minutes. The homogenate was diluted as follows: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. 1 mL of each solution was transferred into marked dupicate petri dishes and violet red bile glucose agar was added (12-15 mL).

After the agar had solidified, an overlay of about 10 ml of violet red bile glucose agar (at 44-46°C) was poured over the surface of each plate and solidified, then incubated at 35-37°C for 21±3 hours.

The purple colonies were counted (preferably on plate containing 30-300 colonies) as presumptive enterobacteriaceae.

For presumptive test, at least two typical colonies were streaked onto a violet red bile glucose agar and incubated at 35-37°C for 20-24 hours.

At least two colonies surrounded by a purpose precipitation zone were selected and streaked onto slants of nutrient agar, and stabbed into tubes of glucose salt medium, then covered with sterile mineral oil and incubated at 35-37°C for 20-24 hours. The growth obtained on the slants were carried out for oxidase text as below.

- Place a 6 cm square piece of filter paper into an empty petri dish and add three drops of tetramethyle-paraphenylenediamine dihydrochloride solution to its centre.

- With an inoculating loop smear cells thoroughly onto the reagent impregnated paper in a line 3-6 mm long. The oxidase test is positive if transferred cells turn dark purple in 5-10 s. If the oxidase test is negative and the glucose salt agar turned yellow the presence of enterobacteriaceae is confirmed.

b) Whole shrimp

50 g of whole shrimp was weighed into a sterile Erlenmeyer flask. To this, 450 mL of peptone solution (0.1%) was added and shaken for 15 min. The solution was diluted until 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} then analysed as the procedure for shrimp gut.

Salmonella determination

Salmonella was determined using original sample of peptone solution (before dilution) as the flow diagram below shows:

Flow diagram of examination procedures for Salmonella

RESUSCITATION

25 g sample + 225 mL buffered peptone water
37°C / 18-24 h

1 mL 1 mL

SELECTIVE ENRICHMENT BROTH

Tetrathionate broth of strontium chloride broth 37°C/18-24 h

Manittol salenite cystine broth. Salenite brilliant green sulphonamide broth 42°C/18-24 h

Plate out

SELECTIVE SOLID MEDIA

Brilliant green sulphadiazine agar

Bismuth sulphite agar

37°C/24-48 h

3 typical colonies / plate

Peptone water

37°C until turbid

BIOCHEMICAL TESTS

Nutrient agar

Lysine decarboxy-lase broth 37°C/18-24 h

ONPG broth

Cled medium

SEROLOGICAL TESTS

RESULTS AND DISCUSSION

Ideally, the gut and shrimp samples for microbiological analysis should be taken from the same product batch. This would be difficult to achieve because most of the raw material that arrives in the factory is already headless. The collection of shrimp gut from the fishermen was not feasable because to collect 50 g of shrimp gut requires large amounts of shrimp, for which no budget was available.

The analytical methods which were used in this work were satisfctory. This was demonstrated by a separate experiment in which shrimp were inoculated with Salmonella, incubated for 24 h at 37°C. Positive results for enterobacteriaceae and Salmonella were obtained.

The results for shrimp gut and shrimp surface are shown in Tables 1 and 2. The enterobacteriaceae test was presumptive for both the shrimp gut and surface. However, in the confirmative test, both shrimp gut and shrimp surface were negative. This indicates that samples of shrimp did not contain enterobacteriaceae.

Table 1

Presumptive and confirmative enterobacteriaceae and Salmonella test results of shrimp gut (Metapenaeus monodon)

Date of sampling	Place of sampling	Case of group of sample	Enterobacteriaceae Presumptive	Confirmative	Salmonella
17-3-87	Pumar Ltd	A		-ve	-ve
7-4-87	Pumar Ltd	B	$51x10^6$-$975x10^6$	-ve	-ve
13-4-87	Pumar Ltd	C	$16x10^6$-$257x10^6$	-ve	-ve
20-4-87	Pumar Ltd	D	$5x10^6$-$27x10^6$	-ve	-ve

A and B both consist of 10 samples each
C and B both consist of 5 samples each

Although the results of the confirmative enterobacteriaceae test were negative, determination of Salmonella were carried out in order to check the confirmative test. The result of Salmonella determination were also negative for all of the samples. Both these test methods were also checked by analysing shrimp samples inoculated with Salmonella and the results were positive for enterobacteriaceae (confirmative) and for Salmonella. Thus the reliability of the methods were demonstrated and the negative results of enterobacteriaceae and Salmonella for shrimp gut and surface were confirmed.

Table 2

Presumptive and confirmative enterobacteriaceae and Salmonella test results of shrimp surface (Metapenaeus monodon)

Date of sampling	Place of sampling sample	Code of group of	Enterobacteriaceae Presumptive	Enterobacteriaceae Confirmative	Salmonella
2-9-87	Serang Coast	A	$6x10^5$-$26x10^5$	-ve	-ve
28-9-87	Cirebon Coast	B	$5x10^5$-$40x10^5$	-ve	-ve
26-10-87	Cirebon Coast	C	$6x10^5$-$30x10^5$	-ve	-ve

A consists of 8 samples
B consists of 12 samples
C consists of 10 samples

Normally, Salmonella is not found in seawater because of the salinity, therefore Salmonella should not be found in the seawater shrimp. It can be found, through, on the shrimp as a result of contamination during handling, transportation or processing and the contamination would not be in the gut of the shrimp but on the surface.

In this case, the samples were not contaminated by enterobacteriaceae including Salmonella, probably because all of the whole shrimp samples were collected at the coast from the fishermen directly (just after being landed).

CONCLUSIONS AND SUGGESTIONS

From this work it can be concluded that:

a) Salmonella was not found in the gut or on the surface of any of the shrimp samples. This indicates that Salmonella is not found in the sea water shrimp naturally.

b) Any Salmonella in the sea water shrimp is probably due to contamination during handling, transportation or processing and thus it must be via the surface of the shrimp.

From this work it can be suggested that in the future, research shold examine methods to prevent or kill Salmonella associated with shrimp; for example the use of chlorine may be useful.

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REPORT ON THE FAO PROJECT ON SHRIMP HYGIENE AND QUALITY CONTROL

by

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ABSTRACT

During the period under this report 11 samples of raw shrimp, 24 frozen shrimp, 47 samples of seafood contact surface, 21 water samples and 8 samples of ice were monitored for microbial analysis. The detailed nature of the microflora is discussed in this report.

INTRODUCTION

The work on this project was initiated at Cochin during the middle of 1987 and 134 samples comprising of raw shrimp, frozen shrimp, seafood contact surface, water and ice were subjected to detailed analysis to understand the nature of microflora associated with them. Cephalopods (squid and cuttle fish) are also a major commodity exported from India. Samples of cephalopods were also taken and a comparative study was carried out to determine the relative degree of contamination between these two groups, shrimp and cephalopods. The results are summarized in Tables 1, 2 and 3.

The study showed that 63.6% of the raw shrimp, prior to processing, have high counts, greater than 1.06×10^6/g. About 50% of the raw shrimp contained coliforms, however, the occurrence of Salmonella was very small, with only 9% of samples showing the presence of Salmonella. In the case of processed shrimp a considerable reduction in total bacterial count was noted with only 4% of samples showing contamination by Salmonella. No sample showed the presence of Vibrio cholerae.

The cephalopods showed an entirely different picture, with both raw and processed cephalopods showing very low total plate count. Raw cephalopod samples tested showed total absence of coliforms. However, analysis of processed cephalopods showed that 10.5% of samples contained Salmonella. This evidently shows that contamination has taken place during handling or transport. Analysis of utensils, water and ice samples showed the occurrence of Salmonella, however, the relative percentage was very low. As the samples were collected from different places it is evident that the majority of the establishments maintained high sanitation.

MATERIALS AND MEHTODS

Serological type testing of Salmonella strains

The 24 strains of Salmonella isolated from raw shrimps, frozen cephalopods and ice were serotyped and the serotypes were as shown below:

Raw shrimps	No. of strains isolated	- 2 Nos.
	Salmonella cerro	- 2 Nos.
Frozen cephalopods	No. of strains isolated	- 14 Nos.
	Salmonella typhimurium	- 14 Nos.
Ice	No. of strains isolated	- 8 Nos.
	Salmonella bareilly	

Two strains of Salmonella isolated from frozen shrimps are under study.

Table 1

Details of samples anaysed

	Numbers
Raw shrimps	11
Frozen shrimps	24
Raw cephalopods	4
Frozen cephalopods	19
Seafood contact surface	47
Water	21
Ice	8
Total:	134

Table 2

Bacterial quality of seafoods

Sample particulars	Total bact.count more than 1.0×10^6/g	Coliforms more than 100/g	E. Coli more than 20/g	Faecal strepto-cocci more than 100/g	Coagulase positive staphylo-cocci more than 100/g	Incidence of salmo-nella	Incidence of V. cholerae	Incidence of non-aggluti-nating vibrios
	%	%	%	%	%	%	%	%
Raw shrimps	63.6	54.5	0	90.9	0	9.0	0	18.0
Frozen shrimps	20.8	33.3	12.5	45.8	0	4.0	0	29.0
Raw cephalopods	0	25.0	0	50.0	0	0	0	50.0
Frozen cephalopods	0	0	0	26.3	0	10.5	0	5.2

Table 3

Bacterial load associated with utensils, water and ice

Sample particulars	Total bact. count more than 100	samples showing incidence of						
		Coliforms	E. Coli	Faecal strepto-cocci	Coagulase positive staph.	Salmo-nella	V. cholerae	Non-aggluti-nating vibrios
	%	%	%	%	%	%	%	
Utensils/ sq. cm.	*35.5	9.0	0	11.0	4.5	0	0	0
Water/ml	19.0	9.5	0	19.5	0	0	0	0
Ice/ml	12.5	12.5	0	12.5		12.5	0	0

* In this case TBC more than 200 is expressed

HISTAMINE CONTENT IN FERMENTED AND CURED FISH PRODUCTS IN MALAYSIA

by

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ABSTRACT

The histamine contents of various fermented and cured fish products in Malaysia were analysed using the fluorometric determination. A wide range of histamine levels was detected. Cured fish had histamine levels ranging from 1.0 mg% to 85 mg% of fish flesh. Fermented fish products such as `cincaluk' and `belacan' showed high levels of histamine (>50 mg%). This paper also reports on studies conducted on the formation of histamine during the processing of these fermented asnd cured fish products, and discusses the possible causes of these high levels of histamine and the economic implications.

1. INTRODUCTION

Food poisoning incidents resulting from the consumption of spoiled fish, especially scombroid species, have been associated with high levels of histamine (Arnold, 1978). Concentrations of 100 mg histamine/100 g fish flesh could cause clinical illness with symptoms such as flushing of facial and neck areas, feeling of intense heat, nausea, vomiting, diarrhoea, hypotension, headache, palpitations and itching.

Histamine is formed from the action of the enzyme histidine decarboxylase on histidine in fish muscle. Histamine poisoning is a form of intoxication with the onset of symptoms in a very short period of time. Although scombroid fish have been associated with histamine poisoning, other food including chicken and cheese have been reported to cause the poisoning.

Much work has concentrated on the formation of histamine in fresh and canned fish, especially tuna. However, only recently has emphasis been given to the histamine content in cured and fermented fish products. Cured fish products are very popular amongst Malaysians. Various types of cured fish are available in the local market including mackerel, anchovies, sardine, gourami. `budu' is a form of fermented fish sauce, popular amongst the population of the Malaysian east coast. `cincaluk' (shrimp sauce) and `belacan' (shrimp paste) are made from Acetes sp. shrimp and are popular condiments in the west coast of Peninsular Malaysia. `Pekasam' on the other hand is a condiment popular in the northern rice growing areas and is made of fermented fresh-water fish such as tilapia and gourami.

Levels of histamine in these fermented and cured fish products in Malaysia have not been thoroughly investigated. This study was carried out to determine the histamine levels in various fish products available in the Malaysian market.

2. MATERIALS AND METHOD

Samples of fermented and cured fish were obtained from local markets throughout Peninsular Malaysia. The extraction and determination of histamine was carried out according to the method of Taylor (1978). Fish samples (10 g) were homogenised in methanol. Selective extraction was carried out using water-saturated butanol and HCl. The histamine extracted was then complexed with ophthaldehyde and then quantified using the Perkin Elmer Luminescence Spectrometer with excitation wavelength of 360 nm and emission wavelength at 450 nm.

3. RESULTS AND DISCUSSION

Figures 1 and 2 show the histamine content in some Malaysian cured fish products. Results show a wide variation of histamine levels in the samples, ranging from 1.1 mg% to 84.6 mg%. Regulatory limits for histamine content in fish varies between countries. In the United State of America, 20 mg% denotes mishandling of fish while 50 mg% levels indicate `hazard action level'. In Germany and Sweden, 20 mg% histamine in fish results in the rejection of a consignment (INFOFISH, 1987). Using these figures, it was observed that more than 50% of all the samples analysed had more than 20 mg%

histamine which denotes decomposition. Five samples namely mullet, anchovies (large and medium size), sardine and horse mackerel had histamine levels higher than 50 mg%. No samples had histamine content above the 100 mg% toxic level.

It is interesting to note that in general, the histamine content of fish samples obtained from the east coast have histamine levels higher than similar samples obtained from the west coast. For example, anchovies obtained from Penang had 3.7 mg% histamine as compared to those obtained from the east coast which had histamine contents ranging from 38 mg% to 58 mg%. This great difference might be due to differences in the delay of processing these fish. In Penang, anchovies were caught and brined on board the vessel, thus, the period between catching and processing is kept to a minimum. In the east coast, however, fish caught are normally stored on board without any processing and then transported back to shore where they are processed. This delay could range from 24 to 48 h. Inadequate chilling and poor handling resulted in deterioration and contamination of fish which consequently resulted in higher histamine levels in the fish. Bacterial contamination often associated with histamine formation include Proteus morganii, Proteus vulgaris, Klebsiella sp., Vibrio sp. and Hafnia alvei (Reilly and Santos, 1985).

Figures 3 and 4 show the histamine levels in fermented fish products. Results show that levels of histamine in these products are high and need attention. `Budu' contained a range of histamine levels depending on the type of fish used. In most cases, anchovies were used as raw material and showed levels ranging from 6.9 mg% to 25.9 mg%. When scombroid type fish were used, such as sardine and mackerel, levels of histamine were higher (26.7 to 65.7 mg%).

Other fermented products based on shrimp (Acetes sp.) also showed high histamine levels. Cincaluk' which is a kind of shrimp paste showed particularly high levels of histamine (84.5 mg%). The source of contamination in this product could be from other ingredients used in the preparation of cincaluk' which include rice and salt. Methods of storage and marketing of these products could also induce further formation of histamine. In most cases, `cincaluk' is marketed in bottles and is exposed to high daily temperatures. The sample that had a high histamine level of 85 mg% was obtained from a road-side stall where the sample had been exposed to the sun for a long period of time.

A high level of histamine indicates poor handling and processing of fish products which resulted in a high degree of microbial contamination. Inadequate chilling or icing of fish can accelerate deterioration and increase histamine levels in fish (Azudin and Samsuri, 1986). Earlier work showed that the histamine content of hardtail scad and yellow stripe trevally reached 47 mg% and 33 mg% respectively after 24 h storage at room temperature. Delays in the salting of fish resulted in higher histamine content (Reilly, 1985). Figure 5 shows changes in the level of histamine in cured fish during drying. Salting of fish in a saturated brine solution resulted in a constant histamine content in mackerel during drying. Histamine content of fish treated with saturated brine solution, 3% sorbate and untreated were 14, 30 and 52 mg% respectively. Results showed that histamine levels in cured fish can be controlled provided the initial histamine load is low. If fish is allowed to deteriorate as a result of inadequate chilling, improper handling and storage, the high histamine content accumulated prior to the processing of the fish will be carried through to the final product.

4. CONCLUSION

As mentioned, histamine is formed as a result of poor handling and storage of fish. Once histamine is formed, it is carried over to all products using the contaminated fish. Greater care needs to be taken to ensure that the fish is properly iced and processed in the most hygenic manner. Further research needs to be conducted locally to study the formation of histamine during the curing and fermentation of traditional fish products and also to find solutions to reduce the histamine levels in the products concerned.

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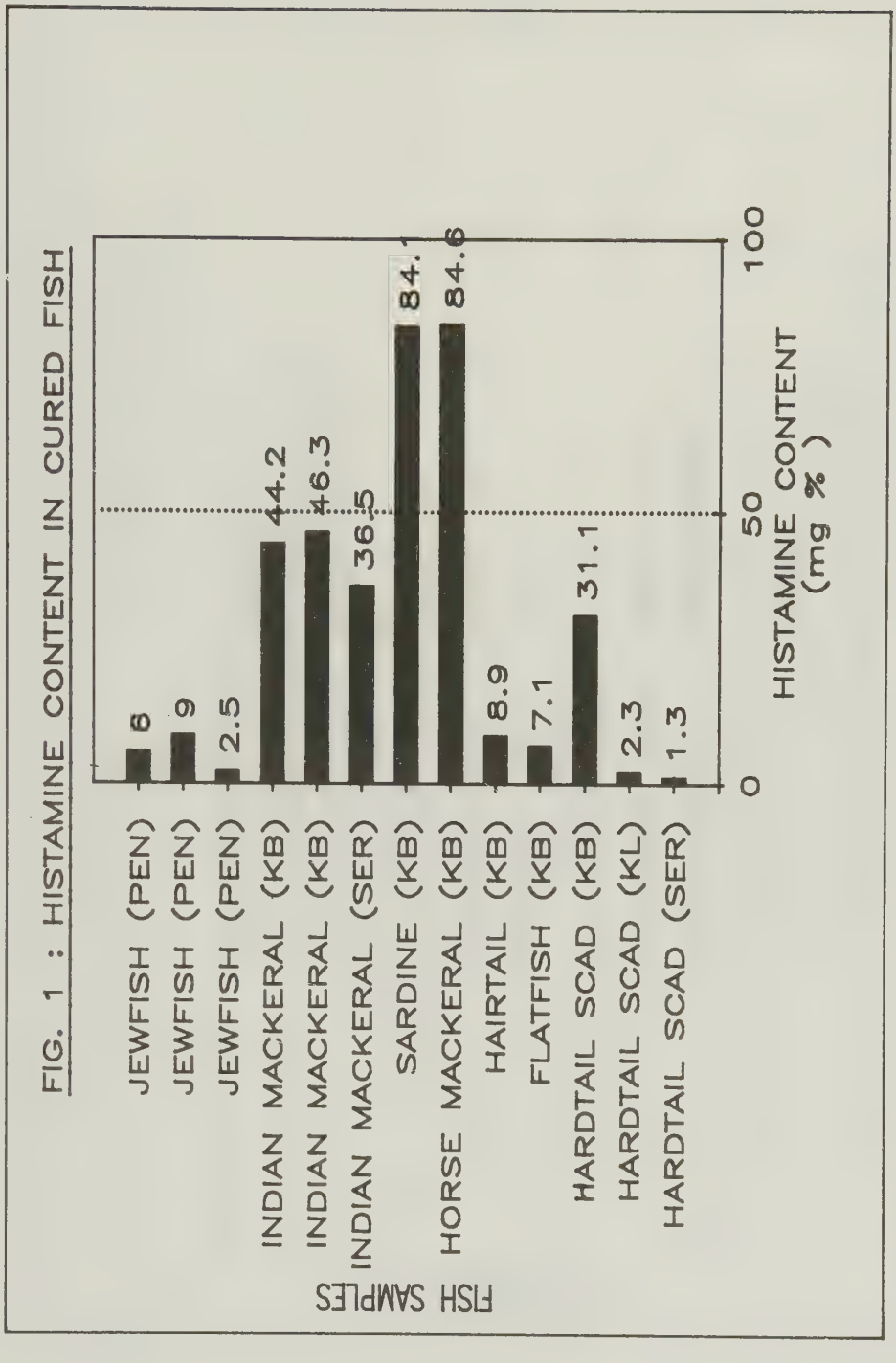
FIG. 1 : HISTAMINE CONTENT IN CURED FISH
FISH SAMPLES
JEWFISH (PEN)
JEWFISH (PEN)
JEWFISH (PEN)
INDIAN MACKERAL (KB)
INDIAN MACKERAL (KB)
INDIAN MACKERAL (SER)
SARDINE (KB)
HORSE MACKERAL (KB)
HAIRTAIL (KB)
FLATFISH (KB)
HARDTAIL SCAD (KB)
HARDTAIL SCAD (KL)
HARDTAIL SCAD (SER)
6
9
2.5
44.2
46.3
36.5
84.1
84.6
8.9
7.1
31.1
2.3
1.3
0
50
100
HISTAMINE CONTENT
(mg %)

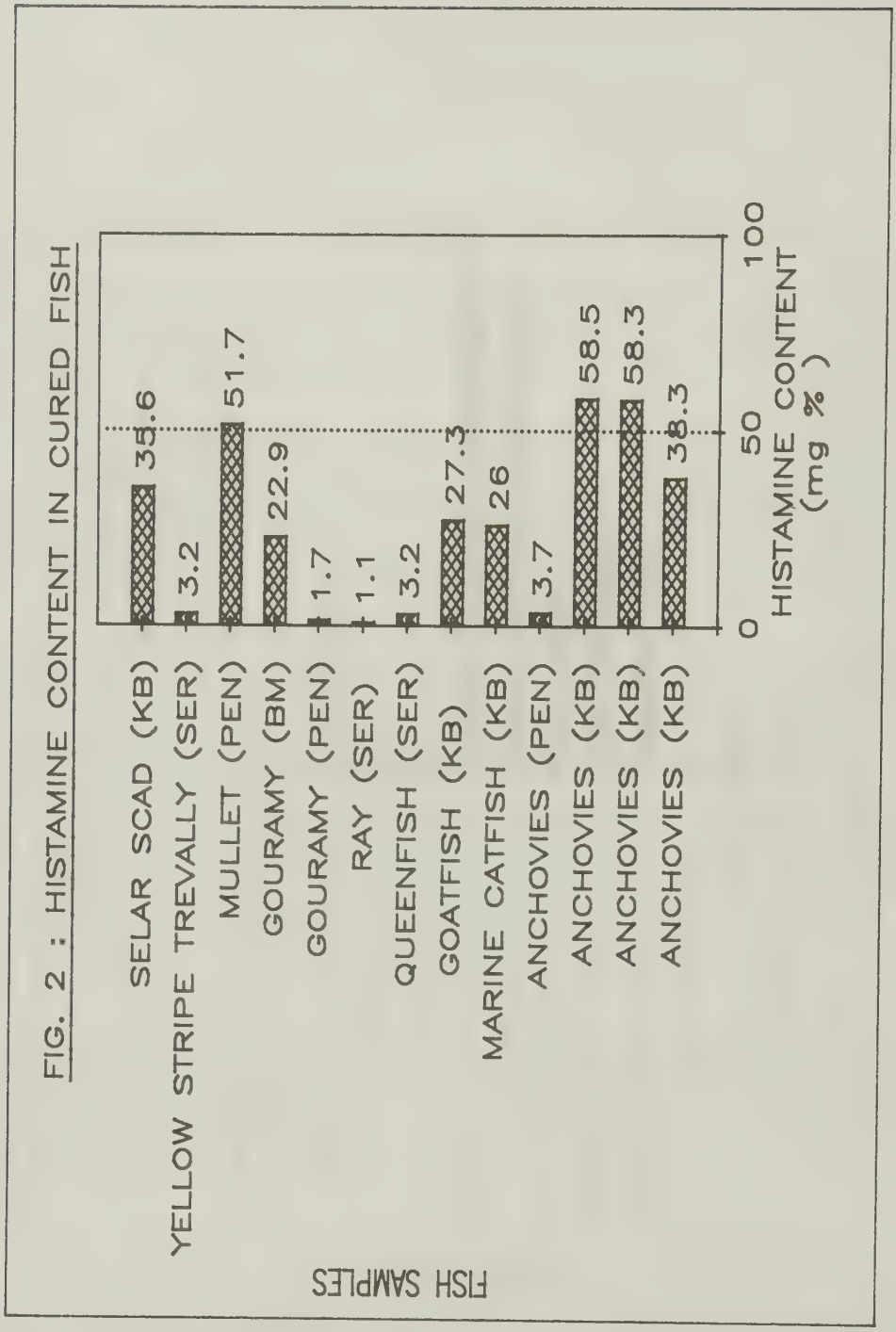
FIG. 2 : HISTAMINE CONTENT IN CURED FISH
SELAR SCAD (KB)
YELLOW STRIPE TREVALLY (SER)
MULLET (PEN)
GOURAMY (BM)
GOURAMY (PEN)
RAY (SER)
QUEENFISH (SER)
GOATFISH (KB)
MARINE CATFISH (KB)
ANCHOVIES (PEN)
ANCHOVIES (KB)
ANCHOVIES (KB)
ANCHOVIES (KB)
35.6
3.2
51.7
22.9
1.7
1.1
3.2
27.3
26
3.7
58.5
58.3
38.3
0
50
100
HISTAMINE CONTENT (mg %)
FISH SAMPLES

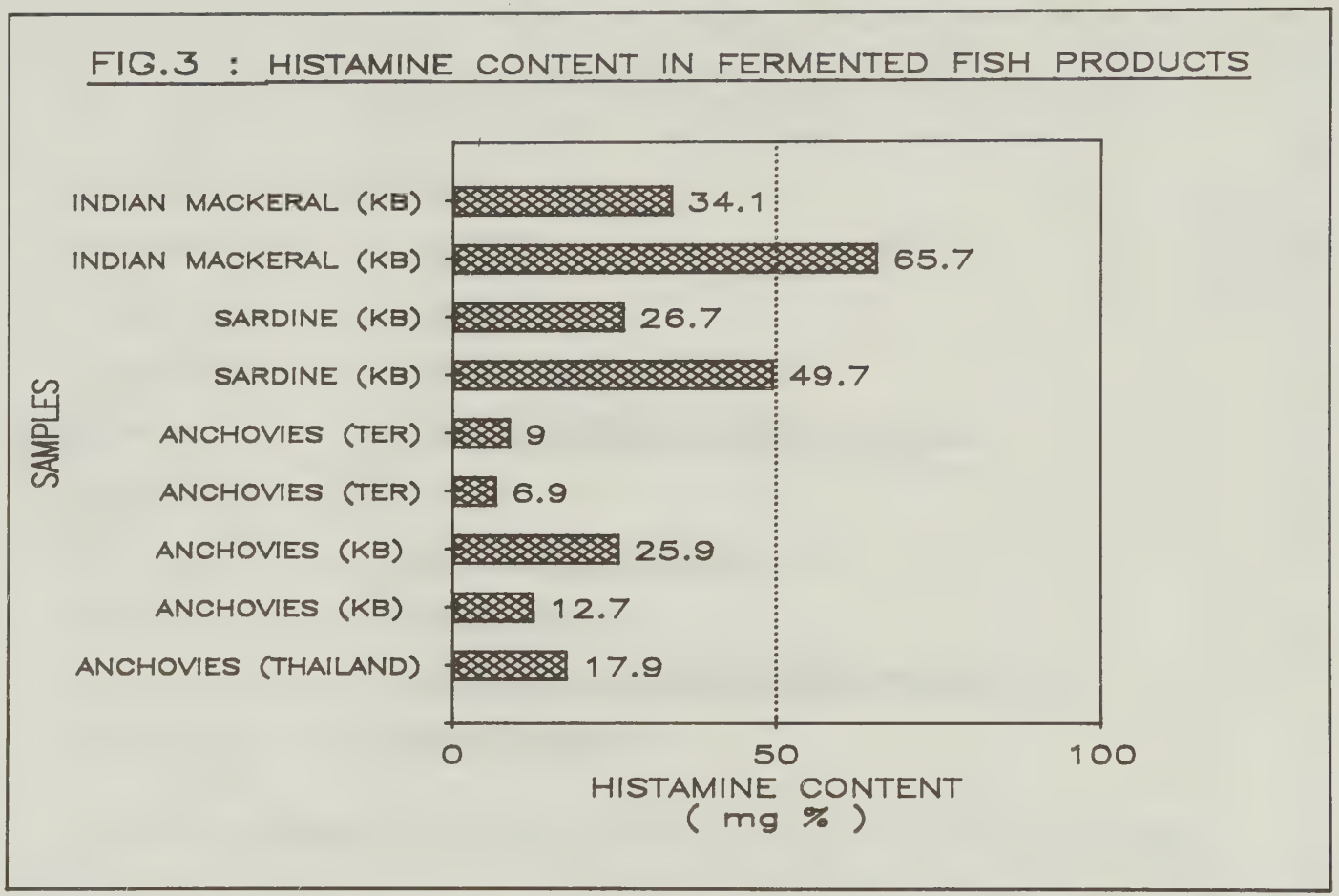
FIG.3 : HISTAMINE CONTENT IN FERMENTED FISH PRODUCTS
INDIAN MACKERAL (KB)
INDIAN MACKERAL (KB)
SARDINE (KB)
SARDINE (KB)
ANCHOVIES (TER)
ANCHOVIES (TER)
ANCHOVIES (KB)
ANCHOVIES (KB)
ANCHOVIES (THAILAND)
34.1
65.7
26.7
49.7
9
6.9
25.9
12.7
17.9
SAMPLES
0
50
100
HISTAMINE CONTENT
(mg %)

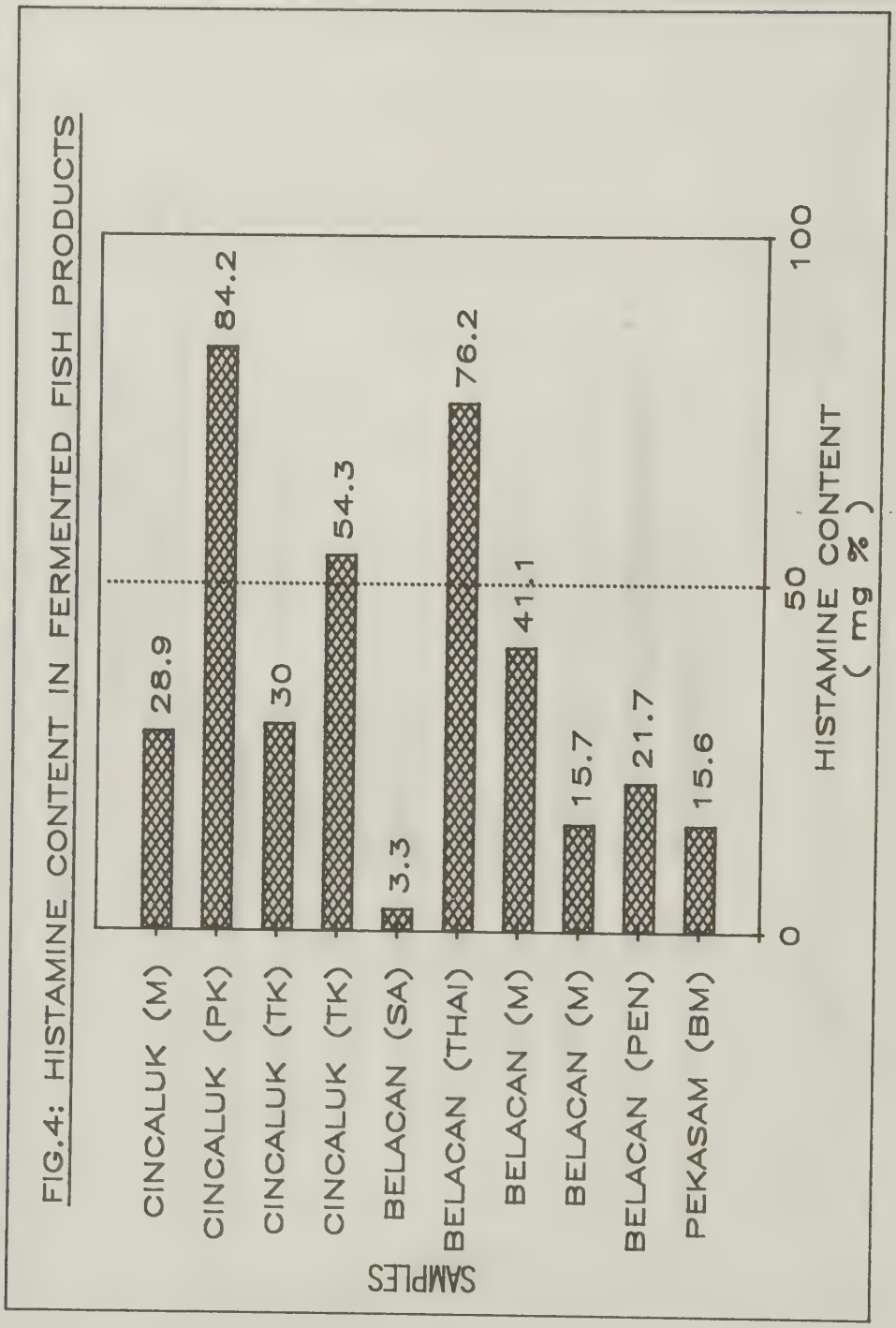
FIG.4: HISTAMINE CONTENT IN FERMENTED FISH PRODUCTS
CINCALUK (M)
CINCALUK (PK)
CINCALUK (TK)
CINCALUK (TK)
BELACAN (SA)
BELACAN (THAI)
BELACAN (M)
BELACAN (M)
BELACAN (PEN)
PEKASAM (BM)
28.9
84.2
30
54.3
3.3
76.2
41.1
15.7
21.7
15.6
SAMPLES
0
50
100
HISTAMINE CONTENT
(mg %)

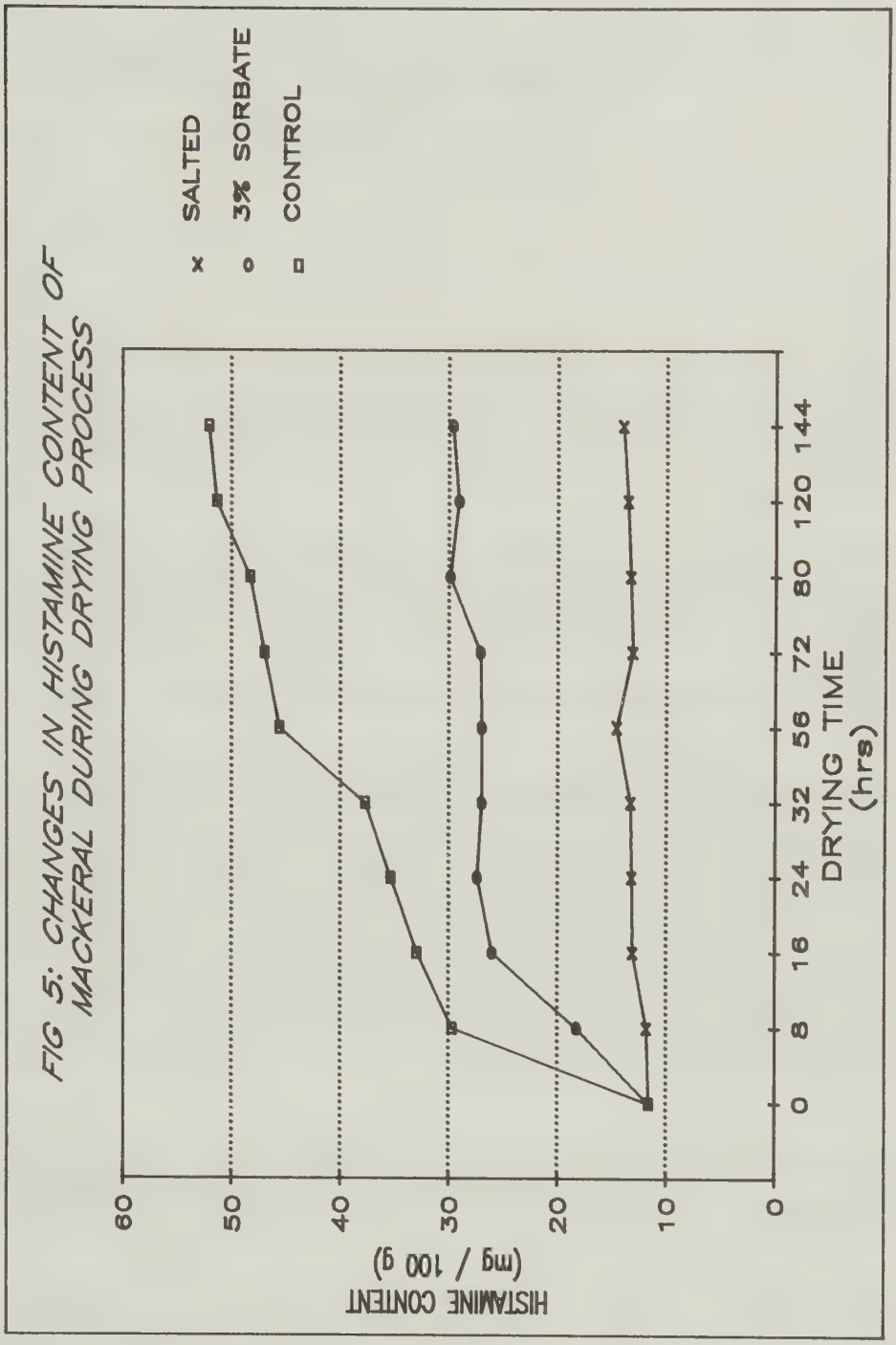
FIG 5: CHANGES IN HISTAMINE CONTENT OF MACKERAL DURING DRYING PROCESS
SALTED
3% SORBATE
CONTROL
HISTAMINE CONTENT
(mg / 100 g)
0
10
20
30
40
50
60
0
8
16
24
32
56
72
80
120
144
DRYING TIME
(hrs)

LEVELS OF HISTAMINE IN SOME OF THE COMMERCIALLY IMPORTANT FISH AND FISHERY PRODUCTS IN INDIA

by

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ABSTRACT

Levels of histamine in a number of raw and processed fish samples were examined in view of concern for public health. Among raw fish, mackerel had levels ranging from 26.55 to 59.85 mg%, sardines 8.31 to 41.56%, seerfish 4.5 to 23.2 mg%, lesser sardines 2.8 to 13.5 mg%. Among processed fish, salted and dried mackerels had high levels (13.3 to 58.5%) and among canned fish, a sample of tuna in oil had 66.5 mg% but canned sardines and mackerels had levels less than 20 mg%.

The levels of histamine in raw fish did not appear to correlate directly with the count of histamine decarboxylating bacteria suggesting that all of them are capable of forming histamine in fish. A study of the microbiological profile of mackerels at various stages of salting and drying did not indicate that histamine formers would proliferate in fish during the initial stages of drying at ambient temperature.

1. INTRODUCTION

It is generally agreed that scombroid poisoning is caused by consumption of fish containing high levels of histamine. Though the characteristics of toxicity are complex and may also involve amines other than histamine, fish implicated in outbreaks of scombroid poisoning have been found to contain histamine at levels exceeding 100 mg% (Arnold and Brown, 1978). Several countries have proposed regulatory limits for histamine in fishes (INFOFISH, 1987) which range from 20 mg% (action level in Sweden; indicative of mishandling in USA) to 50 mg% (hazard action level, USA). About 1.19 million pounds of fish have been reported to have been rejected by US FDA during June-August 1986 due to high histamine levels (INFOFISH, 1987). From India, Subburaj et al, (1984) were the first to report that mackerel marketed in Mangalore had high histamine levels. In this paper we present further studies on levels of histamine in some of the commercially important fish and fishery products marketed around Mangalore and relation between total bacterial count, histidine decarboxylating bacteria and histamine levels.

2. MATERIALS AND METHODS

Depending upon availability, samples of the following fish were collected from the Central Fish Market, Mangalore: oil sardine (Sardinella longiceps), mackerel (Rastrelliger kanagurta), seer fish (Scomberomorus guttatus) and lesser sardines. Samples of salted and dried mackerel were collected from the Dry Fish Market and sardines canned in oil and in brine; mackerel canned in curry and tomato sauce; canned seer fish and tuna were purchased from local retailers.

Levels of histamine in various samples were determined by the method of Hardy and Smith (1976). Total plate count was determined on Plate Count Agar (Hi Media) using the surface spread method described by Speck (1976). Enumeration of histamine formers was done by the method of Niven et al, (1981).

3. RESULTS AND DISCUSSION

In seer fish histamine levels ranged from 4.5 to 23.2mg% (Figure 1). Total plate counts (TPC) ranged from 4.5 to 6 log units and counts of histidine decarboxylating bacteria (HDB) ranged from 3-5 log units, although samples II and III (Figure 1) had very low histamine levels, while this was not to be expected from their TPC and HDB counts.

Sardines had slightly higher histamine levels as compared to seer fish (Figure 2). Four out of seven samples tested had levels exceeding 20 mg%. Sample V had very high levels of histamine though TPC and HDB counts were comparable to that of others. In fact, sample IV had higher TPC and HDB counts compared to sample V but the histamine level in the former was ten times lower.

Mackerel generally had higher levels of histamine compared to other fishes (Figure 3). All the six samples examined had histamine exceeding 20 mg% and half of them had levels around 50 mg%. TPC of mackerel samples were in the range of 5-6 log units and HDB counts in the range 4-5 log units.

As shown in Figure 4, lesser sardines had very low histamine levels, none of them approaching 20 mg%. However, HDB counts were in the range (3-5 log units) found in the other fishes. Even TPC was in the same range (5-6 log units) found in other groups of fishes.

All the samples of salted and dried mackerel examined had histamine levels exceeding 10mg% and six of the eight samples had levels exceeding 20 mg% (Figure 5). TPC in these samples were understandably low (3-5 log units) as also the HDB counts (2-4 log units). Histamine formation in these samples might have taken place before drying and it is possible that many of these histamine formers were eliminated during the process of drying.

Results in Table 1 indicate that 50% of the canned fish examined had histamine levels exceeding 20 mg%. One sample of tuna canned in oil had levels that might be a public health hazard.

The results indicate that histamine levels indicative of decomposition or hazard action level can be found in fish and fishery products marketed around Mangalore. The problem appears to be most acute with mackerel, which are a highly priced fish in this region. Mackerel are caught by both mechanised boats (purse-seines) and non-mechanised boats. Generally, purse-seine catches are iced immediately after landing, but landings from non-mechanised boats may not be iced immediately due to unavailability of ice in villages where these fish are landed. The results are confirmed by Subburaj et al. (1984) who noted higher levels of histamine in mackerel compared to seer fish.

Salting and drying of fish in the region is generally done on a small scale, mainly for local consumption under poor sanitary conditions. Therefore it is to be expected that these products have significant levels of histamine. However, if the average histamine level in salted and dried mackerel (34.76 mg%) is compared with that noted in mackerel from the retail fish market (42.37 mg%), it would seem that during the process of salting and drying, not much histamine is formed. Yunizal et al. (1985) suggested that salt may play an important role in inhibiting histamine formation. However, Hanson et al. (1985) noted that histamine formation could take place during the process of salting and drying. To verify the situation under our conditions, a batch of mackerels having about 30 mg% histamine were salted in the ratio 1:1 overnight and dried under direct sunlight. Results in Table 2 show only a very marginal increase in histamine levels after salting and slight increase during drying which might be only an apparent increase due to moisture loss.

Table 3 suggests that there is no statistically significant correlation between histamine levels and TPC or HDB counts. Similar observations were made by Subburaj et al. (1984). Even the HDB counts did not appear to be a good indicator of histamine levels. Although a number of bacteria are capable of decarboxylating histidine, they have been reported to vary in the rate of histamine production (Arnold and Brown, 1978) and all of them may not be active in fish tissue. Taylor et al. (1978) compared histamine formation by 112 strains of food-borne bacteria and reported that only Proteus morganii and Enterobacter aerogenes produced significant amounts of histamine in tuna infusion. Subburaj et al. (1984) reported that Proteus sp. accounted for 52% of HDB in a sample of mackerel having about 18 mg% histamine. This suggests that it is important to know counts of bacteria such as Proteus morganii and Enterobacter aerogenes rather than HDB counts.

4. ACKNOWLEDGEMENTS

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Table 1

Levels of histamine in some canned fish

Sample number	Product	Histamine (mg%)
1	Sardine in oil	6.65
2	Sardine in natural pack	16.62
3	Mackerel in curry	11.63
4	Mackerel in tomato sauce	19.95
5	Light meat tuna	23.27
6	Tuna in oil	66.50
7	Tuna in curry	26.00
8	Tuna flakes	48.21
9	Seer fish	12.63

Table 2

Effect of salting and drying on levels of histamine and microflora

Parameter	Condition of fish			
	Fresh	After salting	After a day's drying	After two days drying
Histamine (mg%)	31.0	36.50	48.20	52.20
Total plate count/g	9.1×10^4	9.4×10^2	9.8×10^2	3.1×10^2
HDB count/g	1.3×10^6	9.2×10^4	5.6×10^3	3.5×10^3

Table 3

Correlation between histamine level, TPC and HDB counts

Fish	Correlation coeficient between histamine	
	TPC	HDB counts
Seer fish (n=6)	0.34	0.28
Sardine (n=7)	0.04	0.14
Mackerel (n=6)	0.54	0.57
Lesser sardine (n=6)	0.66	0.41
Salted and dried mackerel (n=7)	0.53	0.68

None of the above values are significant at 5% level

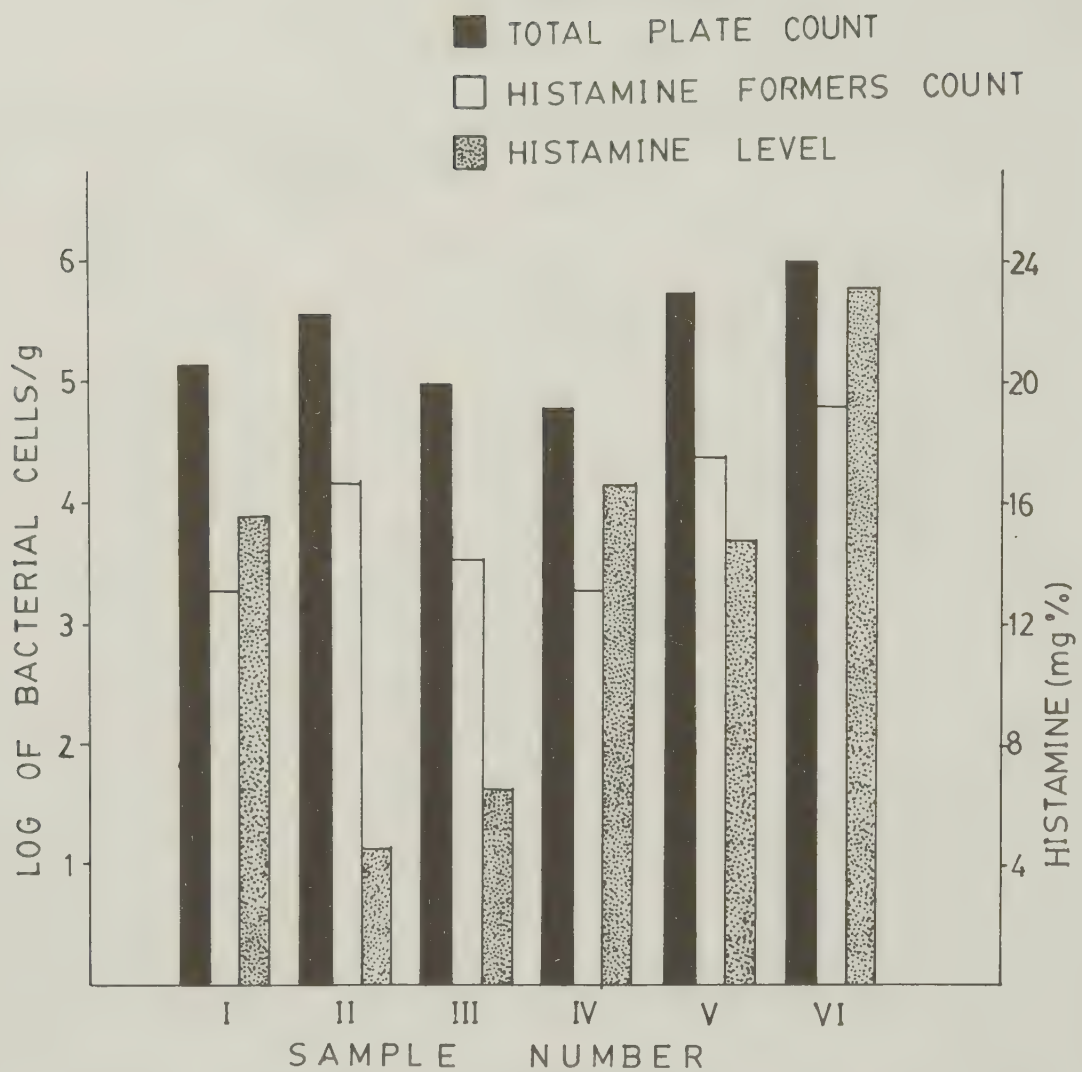

Fig. 1 . Combination of histamine formers, total plate count and levels of histamine in seer fish.

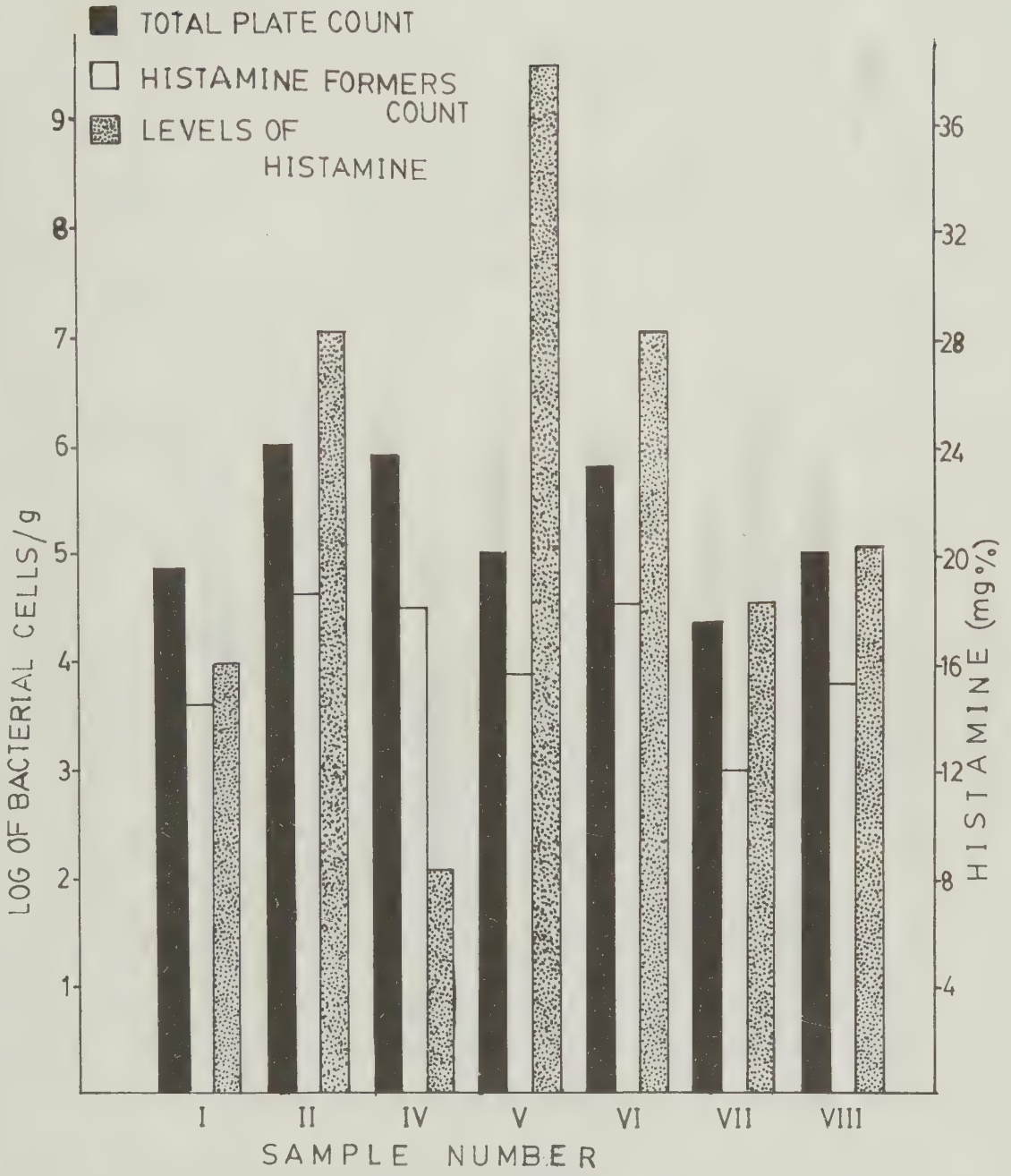

Fig. 2. Relationship between histamine formers, total plate count and levels of histamine in sardine.

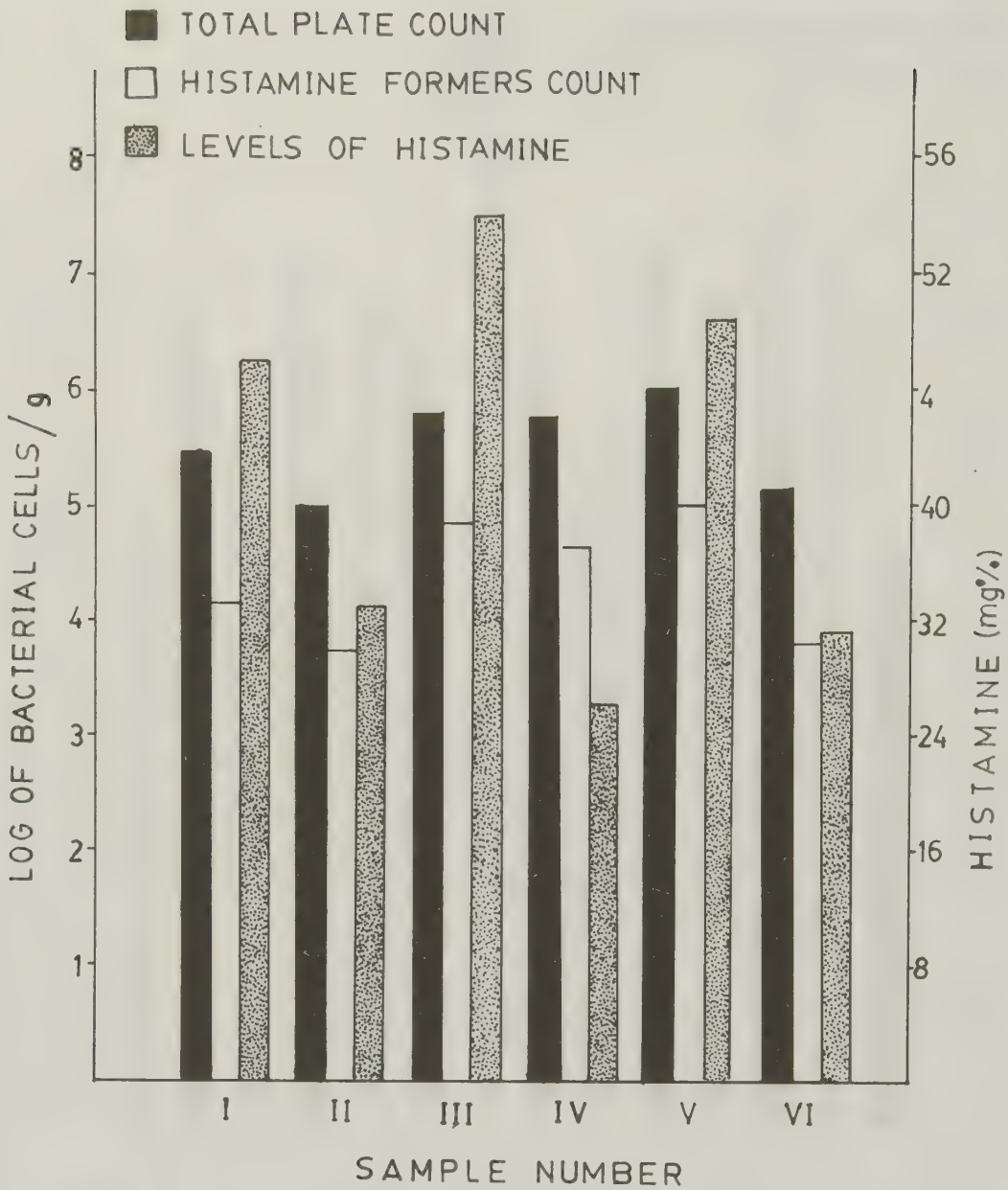

Fig. 3. Relationship between histamine formers, total plate count and levels of histamine in mackerel.

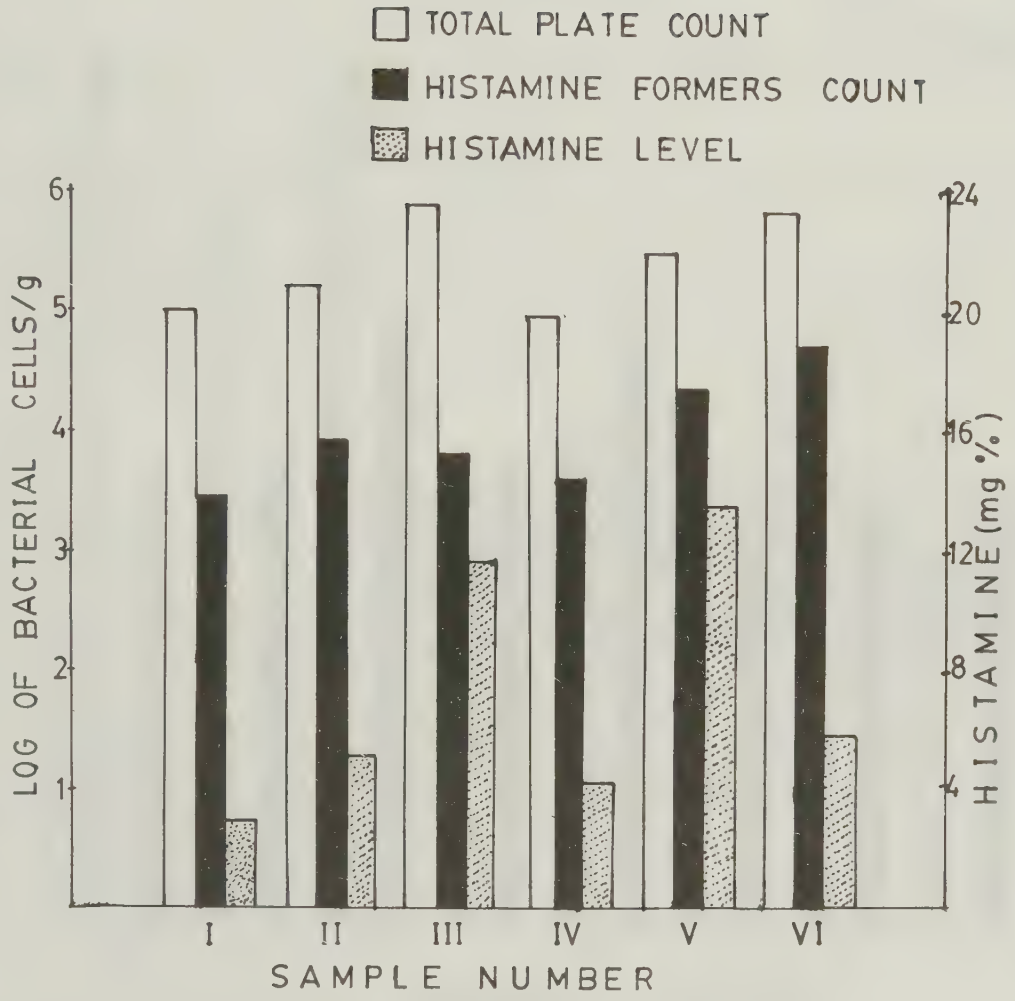

Fig. 4 . Combination of histamine formers, total plate count and levels of histamine in lesser sardines.

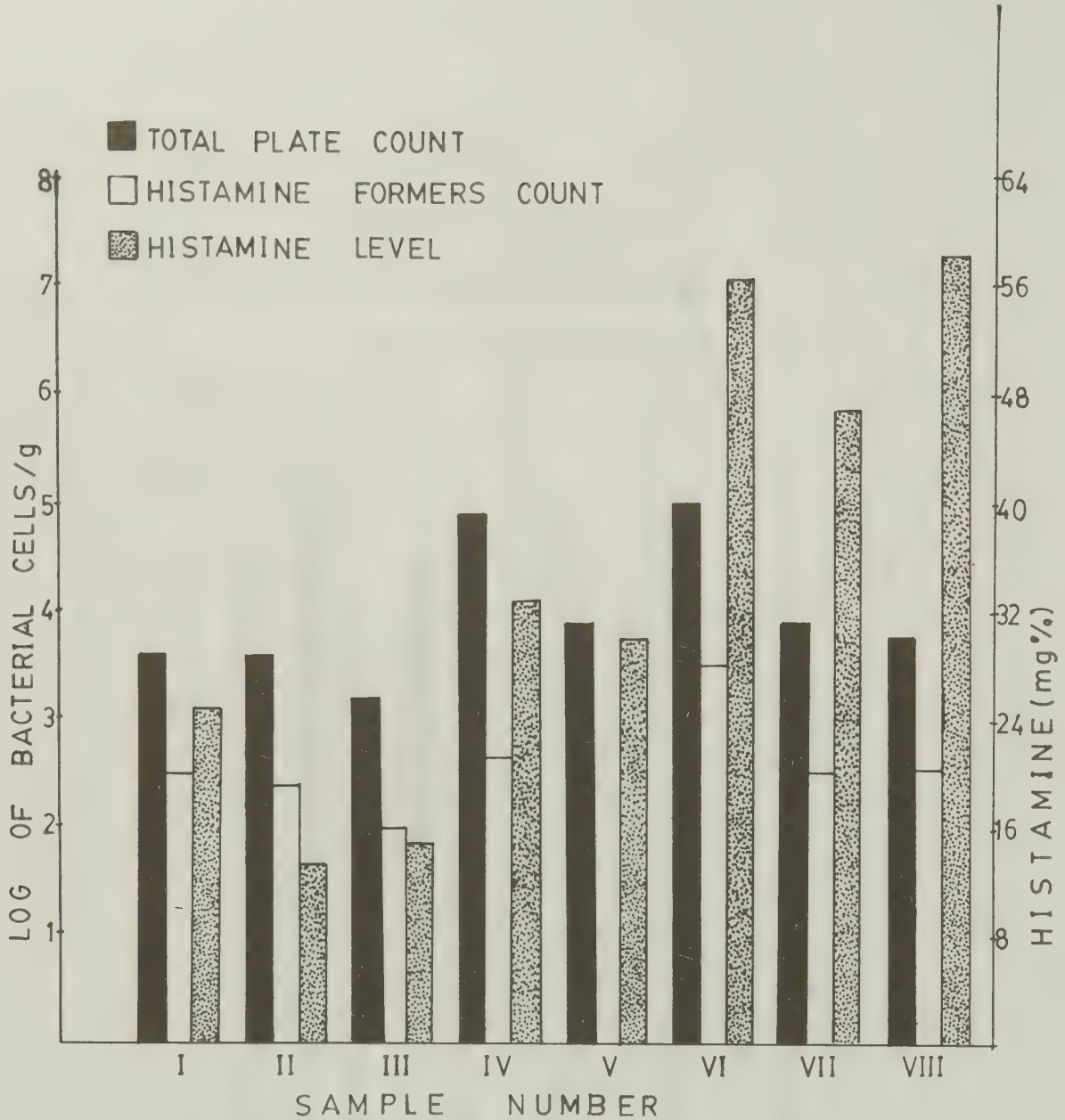

Fig. 5. Combination of histamine formers, total plate count and levels of histamine in salted and dried mackerel.

HISTAMINE IN SCOMBROID PRODUCTS OF THAILAND

by

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ABSTRACT

A study of histamine content of canned scombroid products produced in Thailand was carried out during 1987. Determinations of histamine levels were made by fluorometric method. A total of 607 samples of mackerel, sardine, tuna, bonito, yellow fin and skipjack packed in oil and in brine in chunk and solid pack styles were under investigation. It was found that 100% of mackerel, 95% of sardine, 100% of solid style and 95% of chunk style of tuna contained histamine lower than 50 ppm. Only 2% of chunk style tuna had histamine higher than 100 ppm. The results of this study showed that most of the samples had histamine levels within safety limit.

1. INTRODUCTION

Histamine is the toxic chemical that serves as a mediator of the allergic response. It is formed by the decarboxylation of histidine, an amino acid which occurs in relatively high concentration in the muscular tissue of scombroid fishes (INFOFISH, 1987).

In Thailand, a great variety of canned scombroid products are produced for domestic consumption as well as export. The Division of Food Analysis, Department of Medical Sciences, Ministry of Public Health is responsible for the certification of food for export. Scombroid products were sent for histamine determination to assess their quality.

In 1987, six-hundred and seven samples of scombroid products (in different styles of packing) from many companies were investigated and studied for levels of histamine content. The results of the study will be used to improve the quality of scombroid products in future.

2. MATERIALS AND METHODS

2.1 Materials

A total of 607 samples consisted of:

1. 401 samples of chunk style of various species of canned tuna.
2. 50 samples of solid style of various species of canned tuna.
3. 98 samples of canned sardine.
4. 58 samples of canned mackerel.

2.2 Method of Analysis

Determinations of histamine were carried out by Fluorometric Method (AOAC, 1984).

3. RESULTS AND DISCUSSION

It was found that histamine levels ranged from 0 to 200 ppm. The number of samples in each range were calculated and expressed as per cent of total number of each style.

The histamine levels in chunk style tuna, bonito, yellow fin and skipjack in oil and in brine are shown in Table 1.

Total number of samples were 401. Two-hundred and forty-nine samples or 62.1% were in the range lower than 10 ppm. Three-hundred and eighty-one samples or 95% were lower than 50 ppm. Three per cent of samples were between 51 and 100 ppm, and 1.5% were in the range 101 to 200 ppm. It was found that only 0.5% or 2 samples had histamine content higher than 200 ppm (218 & 294 ppm).

The histamine levels in various kinds of tuna in solid style were similar to those levels for chunk style tuna. There were 50 samples in total and 36 samples or 72% were lower than 10 ppm, while all samples were lower than 50 ppm (Table 2).

There were 98 samples of canned sardine and 64 samples or 65.3% of samples were lower than 10 ppm, while 93 samples or 95% were lower than 50 ppm and all samples were lower than 100 ppm (Table 3).

There were 58 samples of canned mackerel and 43 samples or 74.1% were lower than 10 ppm and all samples were lower than 50 ppm (Table 4).

Tables 1, 2, 3 and 4 show the percentage distribution of histamine levels in chunk style and solid style tuna, canned sardine and canned mackerel respectively.

The results of this study showed that 100% of mackerel, 95% of sardine, 100% of solid style and 95% of chunk style of various kinds of tuna contained histamine lower than 50 ppm. All of the sardine samples were lower than 100 ppm. Only 2% of tuna in chunk style had histamine content higher than 100 ppm.

The good quality of raw material, proper handling and processing are needed for the improvement of end-product quality.

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Table 1. Histamine levels in various kinds of tuna in chunk style

Histamine range (ppm)	Total no. of sample	Per cent	Tuna		Bonito		Yellowfin		Skipjack	
			In oil	In brine	In oil	In brine	In oil	In brine	In oil	In brine
0-10	249	62.09	113	34	77	15	1	5	4	-
11-20	64	15.96	25	9	20	6	1	2	1	-
21-30	37	9.22	16	5	13	2	-	1	-	-
31-40	14	3.49	8	2	3	-	-	1	-	-
41-50	17	4.24	10	3	3	1	-	-	-	-
	(381)	(95)								
51-60	6	1.5	3	2	-	-	-	-	-	1
61-70	2	0.5	1	1	-	-	-	-	-	-
71-80	3	0.75	2	1	-	-	-	-	-	-
81-90	1	0.25	1	-	-	-	-	-	-	-
91-100	-	-	-	-	-	-	-	-	-	-
	(12)	(3)								
101-200	6	1.5	6	-	-	-	-	-	-	-
201	2	0.5	2	-	-	-	-	-	-	-
Total	401	100	187	57	116	24	2	9	5	1

Table 2. Histamine levels in various kinds of tuna in solid style

Histamine range (ppm)	Total no. of sample	Per cent	Tuna		Bonito		Yellowfin		Skipjack	
			In oil	In brine	In oil	In brine	In oil	In brine	In oil	In brine
0–10	36	72	14	9	–	5	4	2	2	–
11–20	7	14	3	–	2	1	1	–	–	–
21–30	4	8	1	–	1	2	–	–	–	–
31–40	3	6	1	–	1	–	–	–	–	1
41–50	–	–	–	–	–	–	–	–	–	–
Total	50	100	19	9	4	8	5	2	2	1

Table 3

Histamine levels in canned sardine

Histamine range (ppm)	No. of cans	Per cent
0-10	64	65.3
11-20	14	14.3
21-30	12	12.24
31-40	2	2.04
41-50	1	1.02
	(93)	(95%)
51-60	2	2.04
61-70	1	1.02
71-80	-	-
81-90	2	2.04
91-100	-	-
Total	98	100

Table 4

Histamine levels in canned mackerel

Histamine range (ppm)	No. of cans	Per cent
0-10	43	74.14
11-20	5	8.62
21-30	9	15.52
31-40	1	1.72
41-50	-	-
Total	58	100

INCIDENCE OF HISTAMINE DECARBOXYLATING BACTERIA AND HISTAMINE LEVELS IN FISH SOLD IN RETAIL MARKETS

by

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ABSTRACT

A survey was conducted to isolate the histamine decarboxylating bacteria (HDB) from tropical fish. Among the many species studied, the HDB was detected in 10 samples of fish at appreciable levels. The study also showed that the incidence of histamine is not a serious problem in fish sold in the market as all samples contained levels much below the recommended levels (USFDA) for fresh fish.

INTRODUCTION

Histamine, the causative agent of the so-called `scombroid poisoning', is usually found in high levels in spoiled scombroid fish such as tuna, skipjack, mackerel and other marine fish such as mahimahi (Coryphaena hippurus) (Frank, 1985;). Recent studies (Ames, Gopakumar and Vijayan, 1987) have shown that, in the pelagic fish, oil sardine (Sardinella longiceps) from tropical Indian waters, substantial amounts of histamine were formed, when the fish was stored in higher ambient temperatures of 16°, 23° and 31°C. Also, this occurred before the fish became putrid, while it appeared edible or just edible.

In fish muscle, histamine is formed by the action of histidine decarboxylating bacteria on the amio acid histidine. The fish species, which are implicated in `histamine poisoning', are known to contain high levels of free histidine in their muscle (Kimata, 1961, Ferencik, 1970, Edmunds & Eitenmiller, 1975). Many species of bacteria are able to form histamine from histidine. The rate of production of histamine depends on many factors, particularly their optimum temperature of growth. The major bacterial species implicated in histamine formation in fish belong to the genera Proteus, Enterobacter, Klebseilla, Clostridium and Vibrio (Ferencik, 1970; Niven et al, 1981; Yoshinaga and Frank, 1982; Omura et al, 1978).

Even though extensive work on histamine formation in fish has been carried out in many countries, particularly from the temperate regions (Ferencik et al, 1961; Taylor, 1983; Ferencik, 1970; Arnold et al, 1980; Yoshinaga and Frank, 1982; Salguero and Mackie, 1979; Behling and Taylor, 1982; Hardy and Smith, 1976), only limited work has been reported from India (Ames et al., 1987). In the present studies, an attempt has been made to investigate whether the fish sold through retail outlets in India are safe with respect to histamine content, as well as their content of histidine decarboxylating bacteria.

MATERIALS AND METHODS

Marine fish, commonly used for human consumption were collected from the retail markets of Cochin (a city situated on the south west coast of India). In the laboratory, the fish muscle was analysed for the bacterial profile, including histidine decarboxylating bacteria and histamine content.

The total viable bacterial count (TPC) was determined by pour plate method using tryptone glucose agar (TGA). The plates were incubated at 28±2°C (RT) for 48h and counts taken. Total coliforms and E. coli were determined by the three tube MPN method of the FDA (1978). Faecal streptococci were enumerated using KF agar (FDA, 1978). Histidine decarboxylating bacterial (HDB) count was determined using the differential plating medium of Niven et al. (1981). The plates were incubated at 37°C for 72 h and the purple colonies with a purplish halo were counted as HDB. The histidine decarboxylating bacterial cultures from plates were isolated at random and were identified by the method of Surendran and Gopakumar (1981) and Buchanan and Gibbons (1974).

The differential medium (Niven et al., 1981) without agar and bromocresol purple was used as histidine nutrient broth (HNB) for determination of histamine production by a limited number of the isolated cultures, at three different temperatures, (28±2°C, 15°C and 0°C). Also, histamine formation by these cultures was determined in a fish meat medium, made by incorporating minced

Indian mackerel (Rastrelliger kanagurta) muscle (10% w/v in a 0.5% NaCl solution in distilled water and sterilised at 1.05 kg/cm^2 gauge pressure. Selected HDB cultures were inoculated in the HNB and fish meat media (1 drop of 48 h broth culture). The inoculated tubes were incubated at 28±2°C, 15°C and 0°C for 7 days and the histamine produced was estimated. Histamine content of the fish muscle and the HDB inoculated media was determined by the method of Hardy and Smith (1976), using a Spectronic 21 UVD spectrophotometer (Bausch & Lomb) for measuring the absorbance.

RESULTS AND DISCUSSION

The details of the fish species analysed and their bacterial quality are presented in Table 1. All the fish species analysed were more or less in prime condition and none had a total bacterial count of more than 10^6/g. However, most of the fish harboured coliforms, Escherichia coli and faecal streptococci, indicating contamination from bacteria of faecal origin.

The histamine content of the individual fish of each species, in relation to the incidence of histidine decarboxylating bacteria (HDB) in their muscle is given in Table 2. The population of the HDB in the muscle of these fishes varied from 40/g in the seer fish to 1.12×10^4/g in the Malabar herring. However, the histamine content of the muscle of these fish was less than 1 mg/100 g in all but one of the samples analysed. Even though Lactarius lactarius and Ribbon fish harboured HDB in excess of 10^3/g, their histamine content was zero. While kilimeen (Nemipterus sp.) and Seer fish Scomberomorus sp.) had a histamine content of 0.18 and 0.53 mg/100 g, respectively their HDB count was only 196/g and 40/g respectively. It is noted that all the fish which contained histamine, also harboured HDB, but the opposite was not true.

The FDA has stipulated 20 mg/100 g as the regulatory limit for histamine in fish and fishery products. The levels reported for fish in the present study are very insignificant and unlikely to cause any histamine poisoning to consumers.

Also, these levels are typical of fresh fish and fish stored at low temperatures (approx. 0°C) (Salguero and Mackie, 1979). The low levels of histamine in these fish, in spite of the incidence of HDB, showed that the bacterial activity had not been enough to degrade the histidine in fish muscle to histamine in sufficient quantities.

Distribution of various genera in the histidine decarboxylating bacterial cultures isolated from various fishes from the local markets of Cochin is presented in Table 3. The major genera of the HDB are Proteus, Escherichia, Klebsiella and Vibrio. Also Pseudomonas and Citrobacter sp. have been isolated. Most of the earlier reports (Frank, 1985) also records similar observations.

A selected number of cultures from the major histidine decarboxylating bacterial groups were used for in vitro studies for evaluating their potential to produce histamine in histidine nutrient broth (HNB) and fish meat media (FM) at differnet incubation temperatures (Table 4). It is interesting to note that almost all the strains produced large quantitites of histamine, in the range of 6.8/µmoles/100 mL to 275/µmoles/100 mL in HNB at 28±2°C. The amount of histamine produced in FM was very much less at the same temperature of incubation. At 15°C, the amount of histamine produced was almost 50% of that produced at 28±2°C, in both HNB and FM. It is very significant to note that, most of the cultures studied did not produce any histamine at 0°C in HNB and none of the cultures in FM. Only two strains of the Proteus sp. and one belonging to the Escherichia produced very small quantities of histamine in HNB at 0°C; but they failed to produce any histamine in FM at 0°C.

Behling and Taylor (1982) in their study using pure cultures of Proteus morganii, Klebsiella pneumoniai, Hafnia alvei, Citrobacter freundii and Escherichia coli, on the production of histamine in tuna fish infusion broth, found that low tempratures limit the production of toxicologically significant levels of histamine in tuna fish infusion broth by these bacteria. Studies of Frank (1985), Ames et al.(1987) and Salguero and Mackie (1979) also revealed that the production of histamine in fish muscle was related to the temperature of storage of fish and at the lower temperatures of iced storage of fish, there is no significant production of histamine in fish. Hence, proper icing is the easiest and most important method of control of histamine formation in fish.

4. ACKNOWLEDGEMENT

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Table 1. Bacterial quality of fishes

Sl. No.	Fish species	TPC per g	Total coliforms per g	*E. coli* per g	Coagulase +ve staphylococci per g	Faecal streptococci per g
1	Jew fish (*Otolithus* sp.)	7.64×10^5	7.85×10^2	Nil	208	9.03×10^2
2	Indian mackerel (*Rastrelliger kanagurta)*	6.93×10^5	7.88×10^2	277	Nil	4.67×10^2
3	Carangid (*Chorinemus* sp.)	4.3×10^5	6.0×10^2	55	3×10^2	2.5×10^2
4	Malabar herring (*Thrissocles* sp.)	1.39×10^6	1.03×10^4	1.8×10^3	47	3.82×10^3
5	Silver belly (*Leiognatus* sp.)	4.75×10^5	2.9×10^3	583	1.25×10^2	8.17×10^2
6	Kilimeen (*Nemipterus japonicus)*	1.95×10^6	2.48×10^4	522	Nil	5.56×10^3
7	Seer fish (*Scomberomorus* sp.)	5.5×10^4	1.9×10^2	Nil	17	47
8	Carangid (Thiriyan)	1.17×10^5	1.14×10^3	200	90	2.0×10^3
9	Lactarius (*Lactarius lactarius)*	1.102×10^6	7.76×10^2	Nil	Nil	1.29×10^4
10	Ribbon fish (*Trichiurus* sp.)	4.7×10^5	6.3×10^2	18	1.4×10^2	400

Table 2

Histamine content of individual fishes of each species: in relation to their content of histidine decarboxy-lating bacteria

Sample no.	Fish	Histamine content mg/100g fish muscle* 1	2	3	4	Histidine decarboxylating bacteria/g
1	Jew fish (Otolithus spp.)	0.15	0.06	0.15	0.41	3.26×10^3/g
2	Indian mackerel (Rastrelliger kanagurta)	0.20	0.14	0.42	0.29	2.18×10^2/g
3	Carangids (Chorinemus spp.)	0.88	0.25	0.81	1.30	4.40×10^2/g
4	Malabar herring (Thrissocles spp.)	0.49	0.18	0	0	1.12×10^4/g
5	Silver belly (Leiognathus spp.)	0.30	0.20	0	0	6.4×10^3/g
6	Kilimeen (Nemipterus spp.)	0.18	0.16	0	0	196/g
7	Seer fish (Scomberomorus spp.)	0.53	0.3	0	0	40/g
8	Carangid (Thiriyan)	0	0	0	0	90/g
9	Lactarius (Lactarius lactarius)	0	0	0	0	3.85×10^3/g
10	Ribbon fish (Trichiurus spp.)	0	0	0	0	2.0×10^3/g

* For each species of the fish, histamine content in four fish estimated separately. But for bacterial count, muscle from the four fish was pooled for the estimation.

Table 3

Distribution of various genera in the histidine decarboxylating bacterial cultures isolated from various fishes from local fish markets of Cochin

Sample number	Bacterial genus	% of the total number of cultures identified
1	Proteus	27
2	Escherichia	17%
3	Klebsiella	11%
4	Vibrio	15%
5	Pseudomonas	9%
6	Citrobacter	5%
7	Unidentified	25%
	Total number of cultures identified	220

Table 4

Histamine production by isolated HDB cultures in histidine nutrient broth (HB) and fish meat media (FM) at three temperatures of incubation. (Histamine values in umoles/100mL of the media)

Culture	Histamine in HNB			Histamine in FM		
	28±2°C	15°C	0°C	28±2°C	15°C	0°C
MHDB1 (Vibrio sp.)	9.6	4.7	Nil	2.2	1	Nil
MHDB2 (Vibrio sp.)	7.6	2.0	Nil	1.46	0.76	Nil
MHDB3 (Vibrio sp.)	11.06	6.1	Nil	1.98	0.60	Nil
MHDB4 (Pseudomonas sp.)	6.8	6.0	Nil	0.78	0.43	Nil
MHDB5 (Klebsiella sp.)	15.0	4.7	Nil	1.32	0.43	Nil
MHDB6 (Proteus sp.)	275	193	2	5.02	4.3	Nil
MHDB7 (Proteus sp.)	55	14	0.8	1.25	0.48	Nil
MHDB8 (Proteus sp.)	21	9	Nil	1.7	0.65	Nil
MHDB9 (Escherichia)	8.3	8	Nil	1.34	0.77	Nil
MHDB10 (Escherichia)	98	98	0.7	3.35	0.85	Nil
MHDB11 (Escherichia)	10.3	7	Nil	1.27	0.34	Nil
MHDB12 (Escherichia)	13.2	5.7	Nil	1.5	0.43	Nil

REVIEW OF STUDIES ON SALTING AND DRYING OF SARDINES

by

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ABSTRACT

A series of studies on salting and drying of sardines has been conducted in both Australia and Indonesia. Pilchard (Sardinops neopilchardus) were salted in saturated brine for 3 days at 25°C. Moisture and salt contents were monitored during salting.

Efforts to improve the traditional processing of dried salted fish also conducted through studies on salting and drying times, re-use of brine and acidification of Indonesian sardines (Sardinella longiceps and S. fimbriata). It was revealed that the prolonged salting and drying times practiced by traditional processors were not necessary to obtain acceptable water activity values. The re-use of brine commonly practiced by traditional processors was proved acceptable up to a certain level of re-use.

Methods of acidification (dipping and spraying) using different concentrations failed to show any significant effect on organoleptic properties of the products. The use of natural acidifiers proved impractical.

1. INTRODUCTION

As a major fish-producing country, Indonesia harvests almost 2.0×10^6 t of fish annually with a maximum sustainable yield of 4.6×10^6 t yearly. Production in 1987 reached almost 2.7×10^6 t. Approximately 50% of the catch is traditionally processed with salting and drying being the dominant process. In some places, like East Java, more than 70% of the catch is salted and dried.

When handled and processed in traditional manner, fish suffers a considerable amount of post harvest loss. Currently, the loss is estimated at approximately 30%. In order to reduce the post-harvest loss through improved practices from the time of capture to the time of retail sale, a collaborative research project between ACIAR (Australian Centre for International Agricultural Research), Australia and AARD (Agency for Agricultural Research and Development), Indonesia was initiated in 1984. Edwards et al. (1984) have outlined the scope, aims, research areas and organisation of the project. The present paper summarises some of the work on salting and drying at Muncar, East Java, and fundamental drying studies at the University of New South Wales.

2. RESULTS AND DISCUSSION

2.1 Salting and Drying Behaviour of Pilchard (Sardinops neopilchardus)

Australian pilchards measuring 19.0-21.0 cm in length and 49.36-78.45 g in weight were salted in saturated brine at 25°C for 90 h. Moisture and salt contents were monitored during salting. The experiment was conducted in 2 replicates. Fish salted for 24 h were then dried using three different sets of drying conditions. The conditions of drying air were 35°C/50% RH, 45°C/30% and 55°C/18% RH. In addition, 2 fish orientations (Figure 1) and 2 air velocities (1 and 3 m/s) were used. The schematic of the experimental dryer is shown in Figure 2. During drying, the weight of fish was monitored continuously.

The results of the salting experiment are presented as salt and water content curves in Figures 3 and 4. The salt and moisture contents were calculated based on non-salt solids. Salting results conformed to the accepted behaviour of fish when salted. The data also fitted the model developed by Zugaramurdi and Lupin (1980). The salting equations for pilchard in this experiment are as follows:

$$St = 11.2*\exp(-0.05t) + 57.5*(1-\exp(0.05t))$$

$$Wt = 242.7*\exp(-0.05t) + 176.3*(1-\exp(0.05t))$$

where St/Wt = salt/water content at time t (g/100 g NSS)

t = salting time at t

The emperical correlations between moisture and salt contents are as follows:

$$S = 180.36 - 0.697W$$

Drying results were presented as a curve of moisture ratio against drying time. It was concluded that a two-term model developed by Jason (1964) could be fitted to the data. The equation is as follows:

$$MR = A*\exp(-t/a) + (1-A)*\exp(-t/b)$$

where:

MR = Moisture Ratio
= (Mt-Me)/(Mo-Me)
Mt = moisture content at t (%db)
Mo = initial moisture content (%db)
Me = moisture content at equilibrium (%db)
t = drying time at t

Constant values of the equations are shown in Table 1.

Statistical analysis on the constant values showed that only A and a values were affected by temperature, while there was no significant difference revealed in b values due to temperature. Therefore only the first term of the equation was affected by drying temperature, and the effect was greatest for the steepest part of the drying curve during the first hours only. This can be seen from Figures 5 to 10. Other treatments did not affect the constant values. This indicates that the salted fish did not have constant period, since if it existed, the air velocity would have affected the drying of fish. The adverse effect of high temperature on quality was also observed, and was indicated by the fish produced which were brittle and easily broken, while the skin easily peeled off.

Table 1

Constant values of drying curves of Pilchard using two-term model and its coefficient correlation*

Drying condition	A	a	b	r
A,1,I	0.76	107.28	5.10	0.998
A,1,II	0.76	116.69	4.91	0.998
A,3,I	0.74	82.18	5.59	0.999
A,3,Ii	0.83	84.53	3.49	0.998
B,1,I	0.60	47.74	4.77	0.999
B,1,II	0.66	45.42	3.93	0.999
B,3,I	0.76	38.03	3.33	0.999
B,3,II	0.70	79.85	5.35	0.999
C,1,I	0.63	46.67	3.81	0.999
C,1,II	0.67	30.01	2.01	0.999
C,3,I	0.66	31.53	3.30	0.999
C,3,II	0.65	37.96	3.24	0.999

* Poernomo (1985)

A = 35°C/50% RH B = 45°C/30% RH C = 55°C/18% RH
1 = 1 m/s 2 = 2 m/s I = orientation I II = orientation II

2.2 Effect of Salting and Drying Time on Water Activity (Aw) and Shelf Life of Fringescale Sardines (Sardinella fimbriata)

Prolonged salting times are widely practiced by traditional processors; usually for 2-3 days depending upon the weather. Studies (Poernomo and Authon, 1985; Poernomo et al., 1988) were done to obtain optimum salting and drying times in terms of Aw and the storage life of dried salted fish. Fringescale sardine were salted in saturated brine at ambient temperature for 8, 16, 24, 32 and 40 h. They were then dried at 40°C and 50°C using a controlled mechanical dryer similar to Figure 1. The air velocity was set at 2 m/s while the relative humidity was not controlled, however, it was noted that the relative humidity was in the range of 57.5-60% for both temperatures during drying. After drying for 4, 8, 12, 16 and 20 h, moisture content and Aw of the fish were measured.

The results showed that final Aw and moisture content were affected by both salting and drying time air temperature. Higher moisture removal rates resulted from higher air temperature, producing an adverse effect on fish appearance and texture. The fish produced by higher drying temperature were brittle, very dry and easily broken, while the skin of the fish was easily peeled. Signs of case hardening was also observed. Table 2 shows the Aw and moisture content of the fish.

For storage trial, fringescale sardine were processed at 3 sets of salting and drying times and these were 16 h salting and 20 h drying, 16 h salting and 4 h drying; 8 h salting and 4 h drying. Salting was done in saturated brine at ambient temperature, while drying was performed at 40°C, 2 m/s and 57.5-60% RH. These sets of conditions gave Aw values of 0.74, 0.86 and 0.91 respectively. The dried fish were then stored at ambient temperature in polyethylene pouches for 21 days. It was shown during storage that fish having higher values of Aw suffered greater reduction in organoleptic properties than fish at lower Aw values. Fish having Aw values of 0.91 and 0.96 were rejected by a group of panelists (5 people) at days 15 and 17.

From these studies it may be concluded that salting time in traditional practices can be reduced to 8 h, the effect of salting ceased thereafter at any level of drying. During sun drying temperatures often reach 35°C and produce an internal fish temperature of 40°C. Thus, based on the results of this experiment, sun drying can be done for up to 2 days (totaling 20 h drying) provided that the weather is suitable.

2.3 Effect of Re-used Brine in the Salting Medium on Dried Salted Oil Sardine (Sardinella longiceps) Quality

Traditional processors often use brine many times over as well as using a mixture of old and new brines to salt their fish. This study investigated the effect of the amount of old brine in the mixture on the end quality of fish.

The mixtures used in the study were as follows:

M1 = 100% new brine, 0% old brine,
M2 = 75% new brine, 25% old brine,
M3 = 50% new brine, 50% old brine,
M4 = 25% new brine, 75% old brine,
M5 = 0% new brine, 100% old brine.

The old brine was obtained from a traditional processor in Kalibaru, Jakarta and had been used approximately 20 times and was 25 days old. The new brine was made at the laboratory and was a saturated solution. Salting was done at ambient temperature for 24 h, and the salted fish were then sun dried for 3 days (totaling 21 h drying) and stored at ambient temperature in woven bamboo baskets.

The study revealed that the higher the percentage of old brine in the mixture the lower the organoleptic score, salt content and pH of the fish, while the total volatile base (TVB) of the fish increased significantly, with an increase in the percentage of old brine in the mixture while the moisture content remained constant. This indicates that old brine in the mixture produced low quality fish by reducing the salt penetration rate and thus inducing spoilage. Figures 11 to 14 show the effect of re-used brine on the appearance, salt content, moisture content and TVB of fish.

During five weeks storage, the TVB of fish salted in fresh brine was practically constant, while that of the other fish increased significantly. Volatile bases are a product of protein breakdown and thus it can be deduced that old brine in the mixture tended to stimulate the breakdown of protein.

Old brine in the mixture also tended to produce a lower score for appearance and discoloration as well as for texture during storage. Moisture content and Aw of fish tended to decrease during storage indicating moisture release to the environment. Total volatile bases of fish increased

significantly during storage indicating protein decomposition. The higher the percentage of old brine in the salting medium, the higher the TVB of the fish. Figures 15 to 20 show the changes in some observed parameters of fish during storage.

Fish salted in a mixture having 75% and 100% old brine had been rejected at week 3 and 4, however, there is a need for further study into the relationship between TVB and organoleptic properties of the fish since it was noted in this study that the panelist undertaking organoleptic assessment still accepted fish having TVB above 125 mgN/100 g. Another study is also necessary to investigate the maximum re-use of brine.

2.4 Studies on the Acidification of Dried Salted Fish

To reduce fly landings on fish during drying, studies on the use of acid have also been conducted. These studies were initiated in Australia using pilchards (Sardinops neopilchardus). Pilchards were salted at 25, 30 and 35°C in saturated brine having three different initial pH (4,7 and 9.4). An acetic acid solution (1%) was used to adjust the pH of the brine. Salt and moisture contents were measured during salting and it was revealed that salt uptake and moisture loss fitted Zugarramurdi and Lupin's model. The constant values are shown in Table 3. Statistical analysis showed that temperature and pH did not significantly affect the constant values at 5% level of confidence. It was also noted that the pH of the brine changed during salting and all ended with similar values (pH 7). This was probably due to the buffering capability of the protein from fish blood and muscle. Some of the salting curves are shown in Figures 21 to 26.

A study into the effect of acidification on the organoleptic properties of the fish was also conducted. Salting in saturated brine was conducted for 10.5 h and then drying was done at 45°C, 30% RH and 2 m/s air velocity. Drying was completed when the moisture content of the product reached 50%. This was achieved by monitoring the weight loss through a balance connected to the drying trays.

An acetic acid solution was used to acidify the fish by dipping the fish prior to drying or spraying the fish prior to the completion of drying. The concentrations of the acid was 1%, 2%, 5% and 10% (v/v). Non-acidified fish was used as a control. Preference tests were done using a hedonic scale scoresheet. Nine Asian students, mainly Indonesian, were chosen and asked to assess the appearance, colour, smell, taste and texture of dried salted fish. For taste and textural assessment, the dried salted fish were cooked. Surface pH was also measured.

The results showed that acidification significantly affected the surface pH of the fish. Statistical analysis showed that the acidification method affected the taste of the fish. Acid levels, on the other hand, affected the taste, texture and colour. The highest average score was gained by the control treatment (5.02 out of 7) followed by the fish dipped in 10% acetic acid before drying (4.82).

Following the studies in Australia, similar studies on acidification were conducted in Indonesia. There were two studies conducted in Indonesia, the use of acetic acid and the use of natural acidifiers (RIFT, 1987 and 1988).

To simulate the traditional process, a traditional processor in Muncar, East java, was asked to salt oil sardines (S. longiceps) in his salting tank and the brine had been used several times. It was noted that the processor used 430 L old brine to which was added 166 kg crystal salt and 499 kg fish. The salting time was 44.75 h. After salting the fish was rinsed and drained prior to drying.

Drying was done in the sun for two days (15.25 h). Approximately 2 h before the drying was completed, a batch of fish was sprayed with 15% acetic acid on both sides. After drying, the fish were piled in trays and kept in the warehouse overnight. The next morning the fish were packed in wooden boxes and road transported to Jakarta. They were then packed in wooden boxes, wooden boxes lined with polypropylene sheet, wooden boxes lined with polyethylene sheet and wooden boxes lined with brown paper and sent to a warehouse in Kalibaru (for storage test) which belonged to a wholesaler.

Observation during drying and an overnight keeping of the fish at warehouse at Muncar showed that the frequency of flies landing was less on the acidified fish than on the non-acidified ones. However, this study failed to reveal the effect of acid and packaging materials on the shelf life of dried fish. All the fish were rejected by panelists at week 3 and 4. Evidence also showed that some insects were repelled from the acidified fish during the first week of storage.

Natural acidifiers such as tamarind (Tamarindus indica) and "belimbing wuluh" (Averrhoa balimbi) are common condiments used by housewives in Indonesia. Studies to examine the possibility of using these acidifiers in fish salting were conducted in Muncar. Chopped tamarind and "belimbing wuluh" (5%, 10% and 15% w/v) were added to the brine of traditional processors. Oil sardines were

salted in this brine for 12 h, sun dried for 3 days, packed in polyethylene pouches and kept at ambient temperature. It was found that there was no effect of natural acidifiers on the quality of dried salted oil sardines. All the fish were rejected by the panelists at days 25 to 30. Other parameters observed, such as moisture content, TVB, pH and Aw were not significantly different between treatments during storage. The use of natural acidifiers again proved impractical.

3. CONCLUSION

Information on salting and drying of sardines has been obtained from studies conducted under an ACIAR-AARD collaborative research project. It was shown that salted fish do not have an apparent constant rate period during drying. The drying of salted fish was affected by air temperature and relative humidity, while air velocity and fish orientation relative to air flow failed to show any effect.

Efforts to improve traditional processing have also been done. Salting by the traditional process can be reduced to 16 h while 50% old brine in the salting medium proved acceptable. Acidification, on the other hand, did not affect the shelf-life of dried salted fish.

More studies on traditional processing are necessary to establish a standardised method of processing to obtain better quality products. Efforts to overcome the climatic problems and constraint in packaging should also be continued.

4. ACKNOWLEDGEMENT

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Table 2

Water activity and moisture content of dried salted fish at various processing conditions[1]

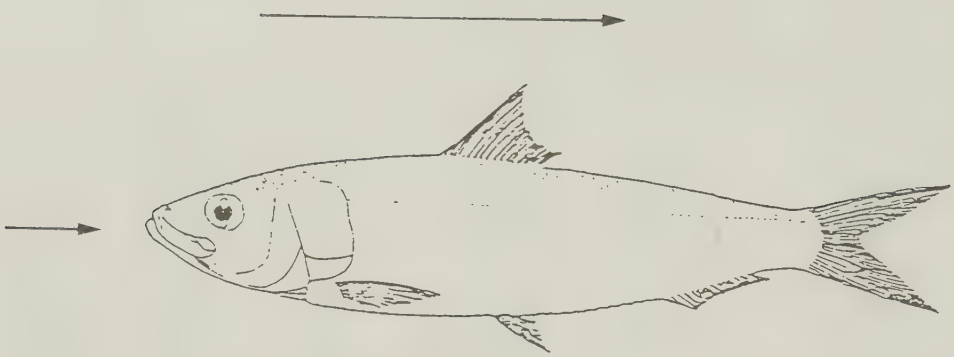
Salting time (hours)	Drying time (hours)	Dried at 40°C		Dried at 50°C	
		a_w	mc*	a_w	mc*
8	4	0.82	57.68	0.80	52.59
	8	0.80	50.71	0.75	43.35
	12	0.76	47.72	0.74	39.02
	16	0.74	41.41	0.74	39.71
	20	0.74	42.33	0.73	30.49
16	4	0.76	52.74	0.75	49.57
	8	0.75	47.88	0.75	44.16
	12	0.75	44.87	0.75	40.88
	16	0.74	42.40	0.75	40.75
	20	0.74	43.11	0.71	35.20
24	4	0.75	51.50	0.75	49.63
	8	0.75	46.40	0.75	44.03
	12	0.75	46.11	0.75	41.25
	16	0.75	41.08	0.75	40.52
	20	0.75	43.58	0.75	34.48
32	4	0.75	49.93	0.75	50.34
	8	0.75	48.36	0.75	45.18
	12	0.75	46.60	0.75	43.05
	16	0.75	43.67	0.75	42.09
	20	0.75	44.36	0.75	41.02
40	4	0.75	50.35	0.75	48.33
	8	0.75	46.68	0.75	44.66
	12	0.75	46.04	0.75	41.99
	16	0.75	44.81	0.75	42.32
	20	0.75	43.92	0.75	41.89

1) Poernomo and Authon (1988)
* % wet weight basis

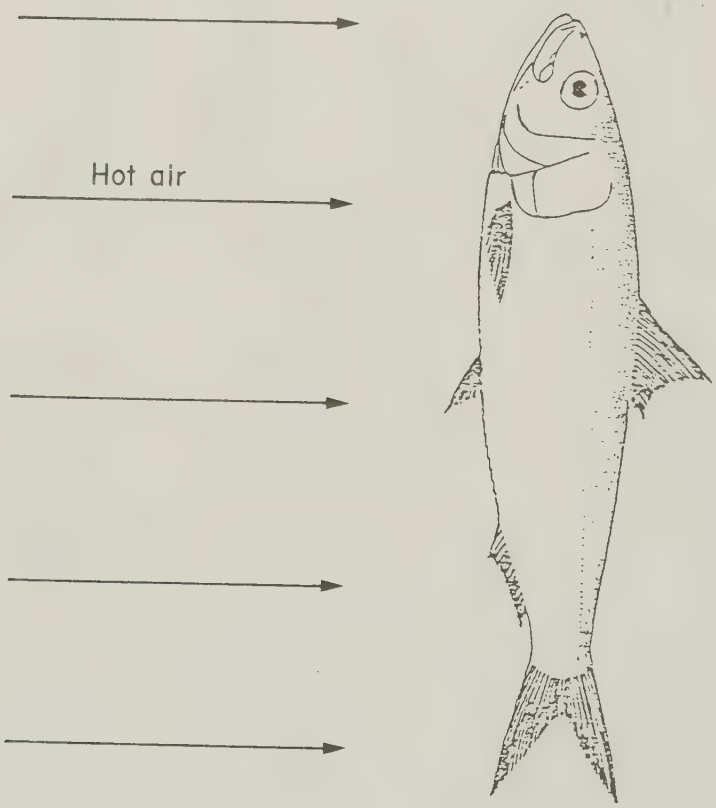
Table 3

Value of Xs, Ks, Xw and Kw from the experimental data fitted to Zugarramurdi and Lupin's model (Utomo 1985)

Temperature	pH	Replication	Salt		Water	
			Xs	Ks	Xw	Kw
25	4.0	1	82.95	0.03	184.15	0.21
		2	51.60	0.09	173.31	0.14
	7.0	1	66.43	0.07	196.83	0.18
		2	63.19	0.07	184.16	0.12
	9.4	1	99.97	0.02	171.81	0.19
		2	68.6	0.07	185.32	0.15
30	4.0	1	68.55	0.087	195.64	0.16
		2	72.98	0.08	205.62	0.22
	7.0	1	77.03	0.08	201.74	0.12
		2	74.23	0.08	191.35	0.17
	9.4	1	73.50	0.09	198.70	0.11
		2	68.70	0.10	195.37	0.10
35	4.0	1	73.05	0.07	202.14	0.14
		2	68.45	0.89	162.46	0.34
	7.0	1	75.51	0.07	196.73	0.12
		2	77.18	0.08	205.26	0.15
	9.4	1	79.38	0.08	206.98	0.11
		2	80.59	0.07	207.48	0.12



Orientation I



Orientation II

Figure 1 Fish orientation

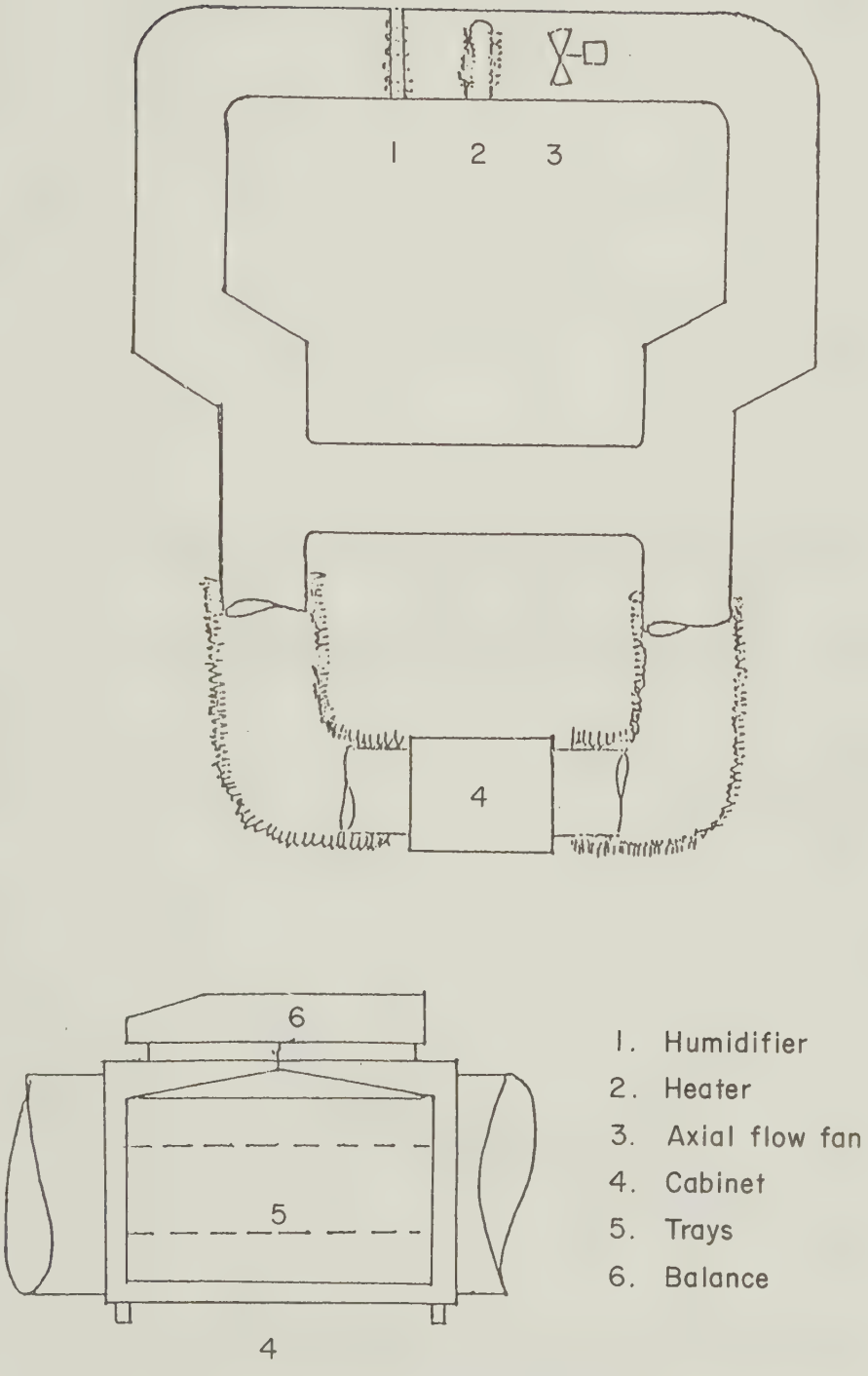


Figure 2 Schematic of dryer used in experiment

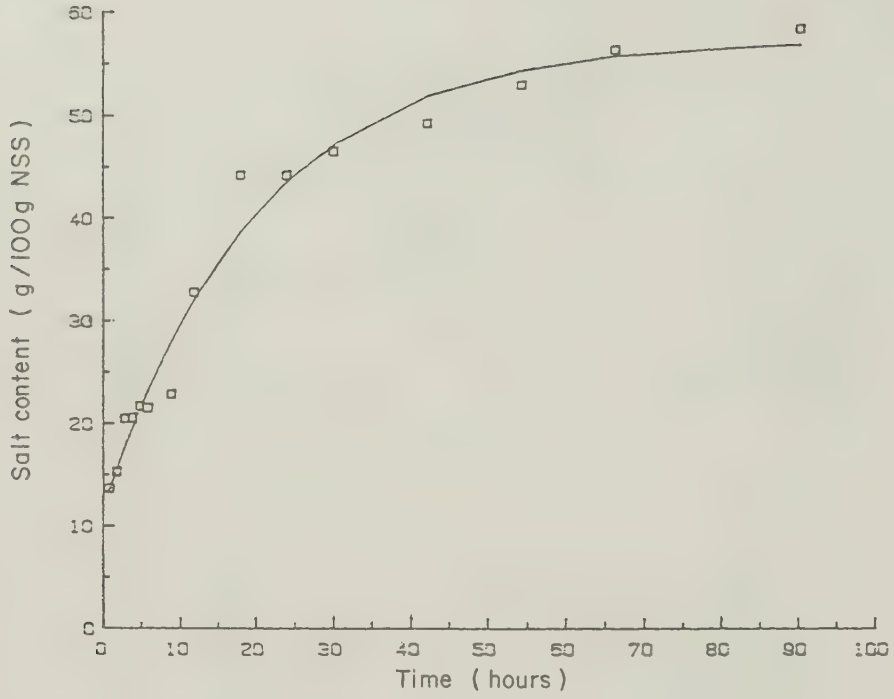


Figure 3 Salt content of sardines during salting

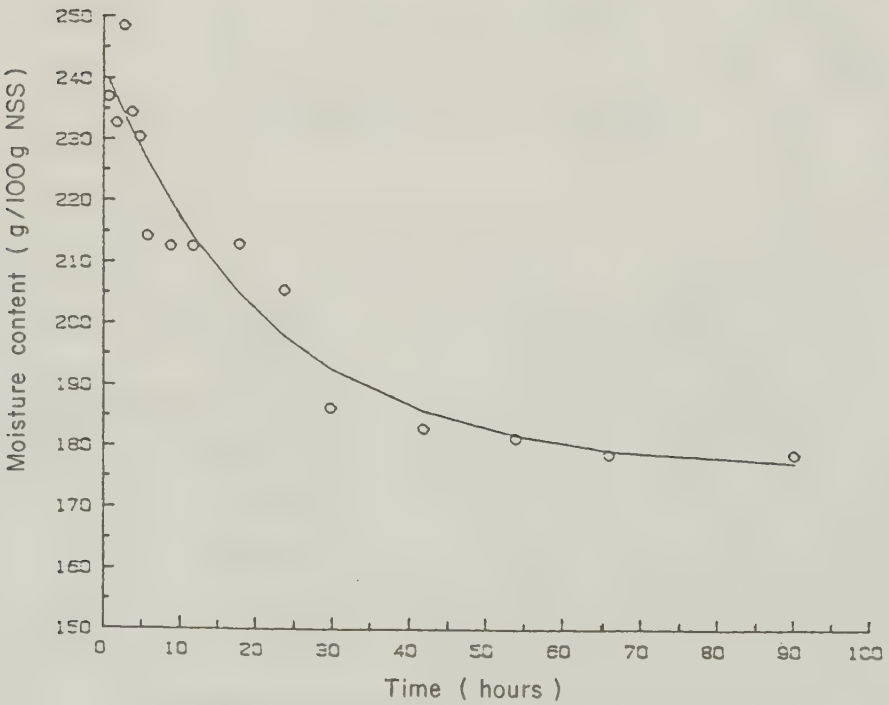


Figure 4 Moisture content of sardines during salting

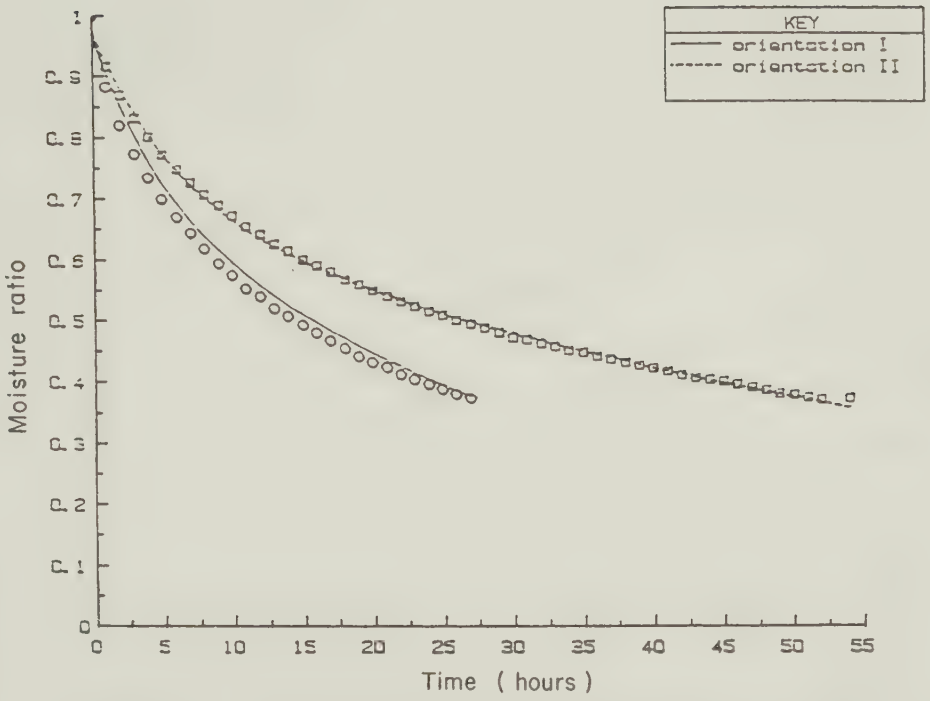


Figure 5 Drying curves of salted sardines at 45 °C and 30 % RH
(3 m/s fitted to three term-model)

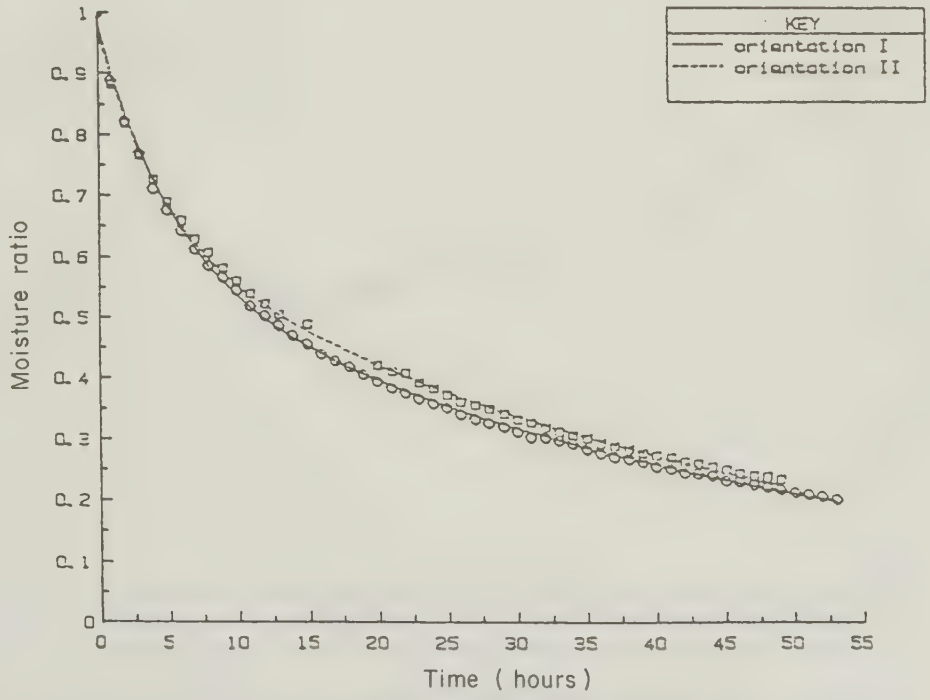


Figure 6 Drying curves of salted sardines at 45 °C and 50% RH
(1 m/s fitted to three term-model)

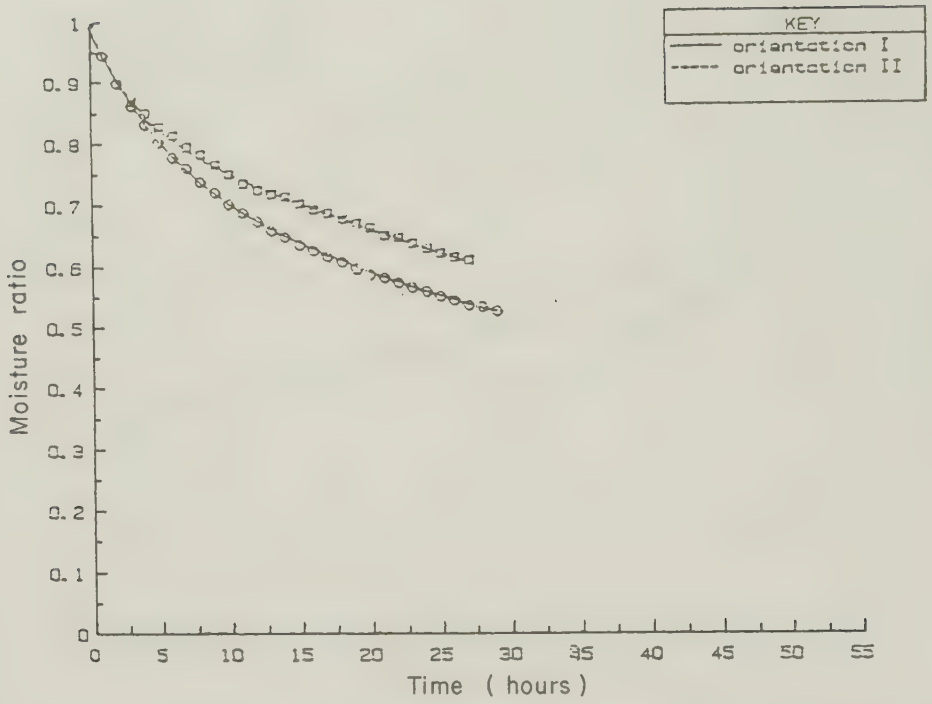


Figure 7 Drying curves of salted sardines at 35 °C and 50 % RH
(3 m/s fitted to three term-model)

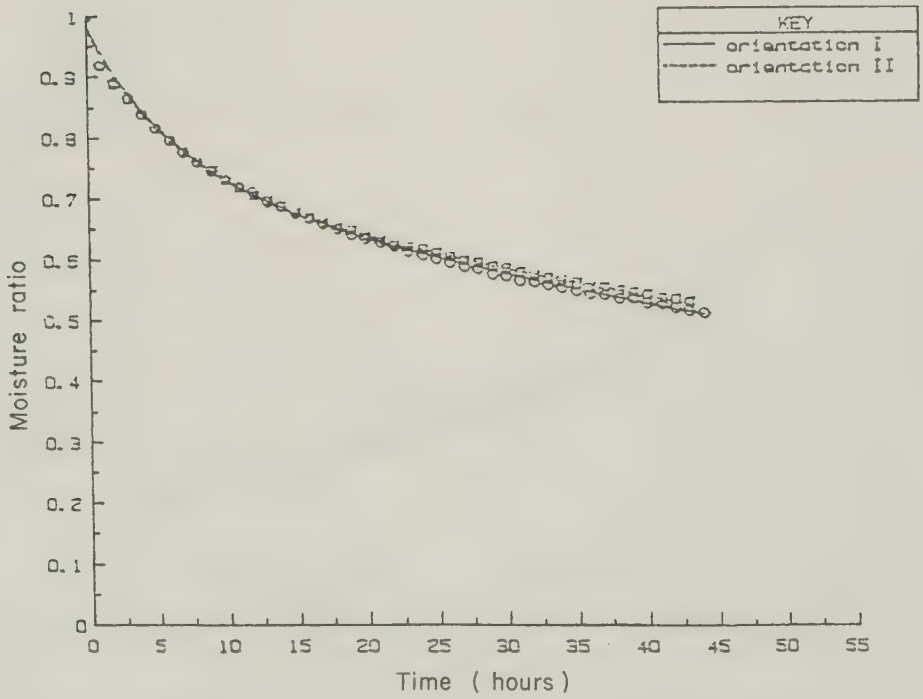


Figure 8 Drying curves of salted sardines at 35 °C and 50% RH
(1 m/s fitted to three term-model)

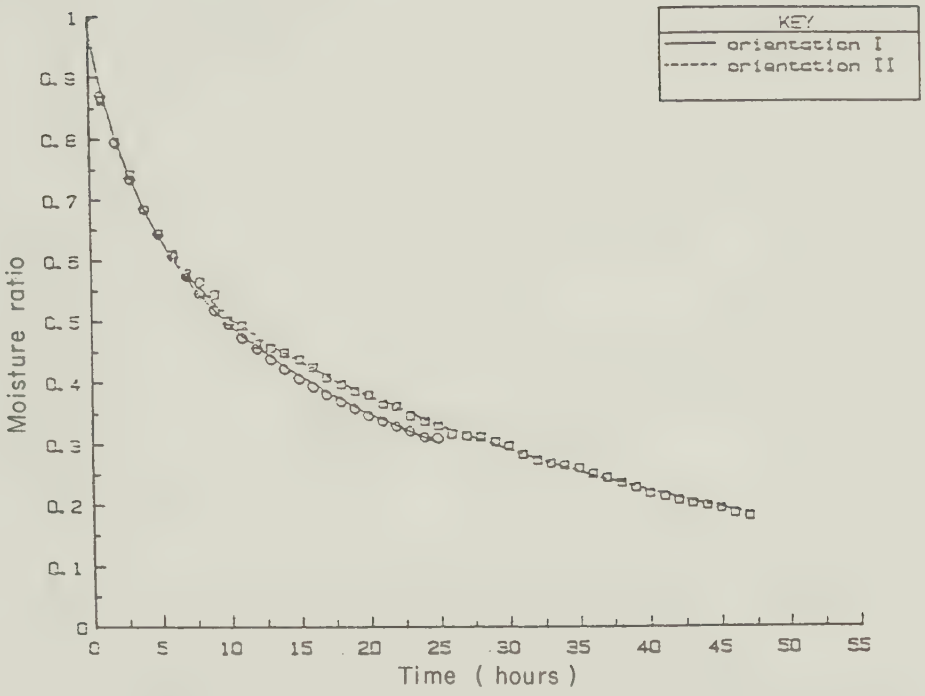


Figure 9 Drying curves of salted sardines at 55 °C and 18 % RH
(3 m/s fitted to three term - model)

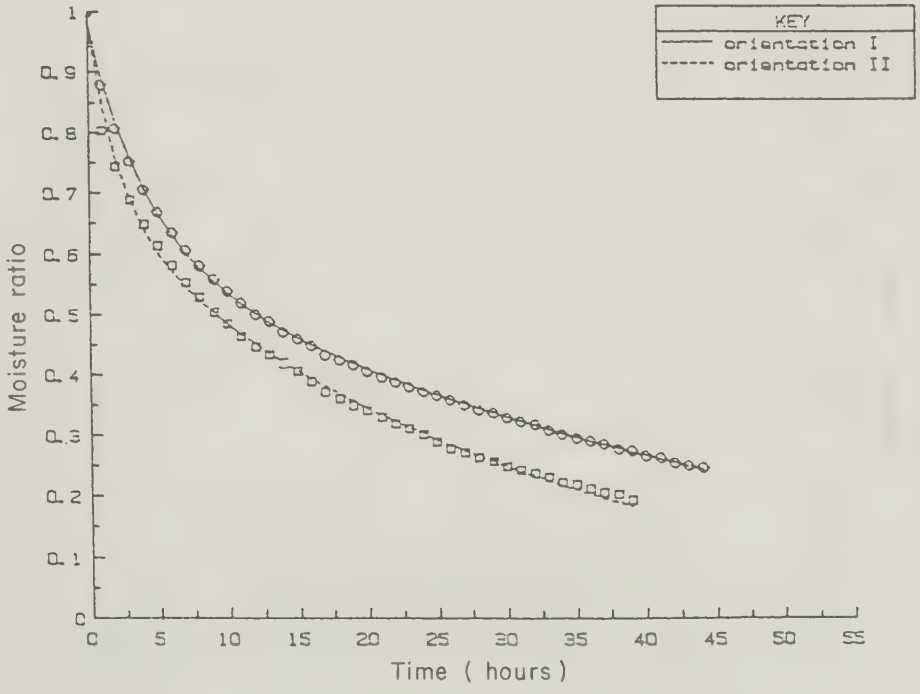


Figure 10 Drying curves of salted sardines at 55 °C and 18 % RH
(1 m/s fitted to three term - model)

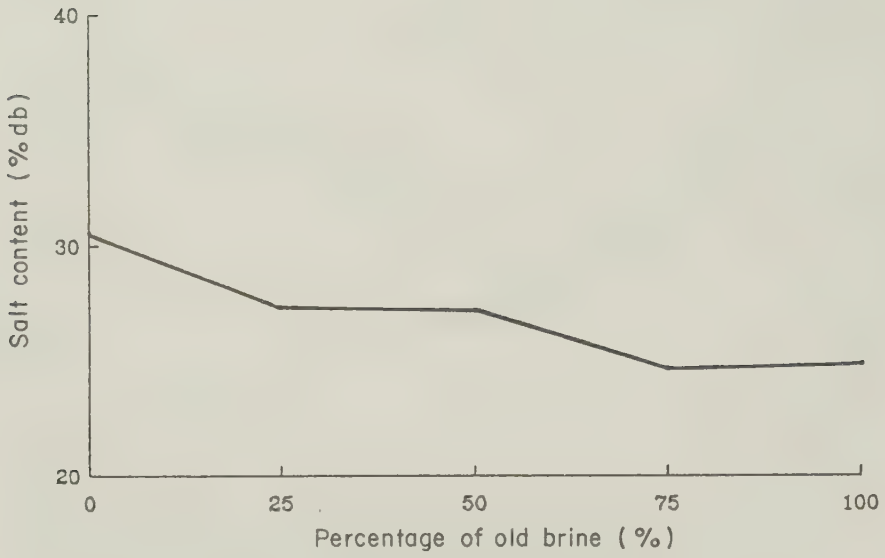


Figure 11 Effect of reused brine on salt content of fish

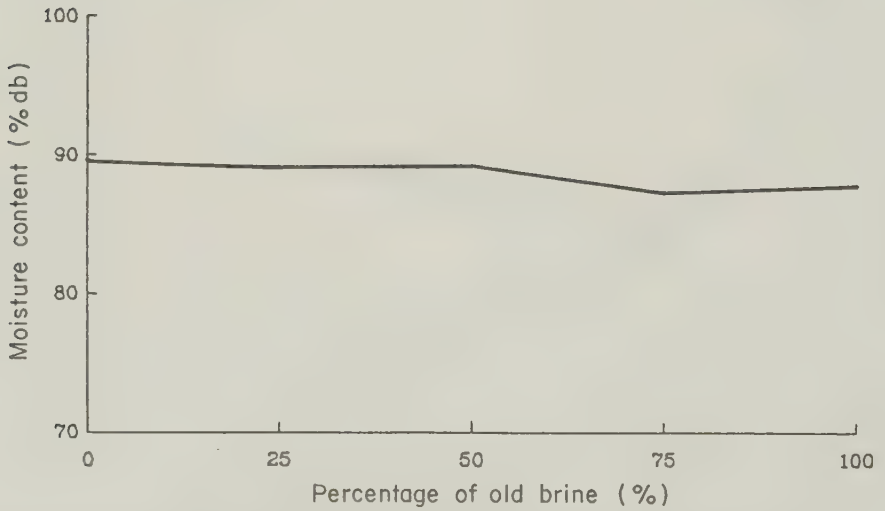


Figure 12 Effect of reused brine on moisture content of fish

Figure 13 Effect of reused brine on TVB of fish

Figure 14 Effect of reused brine on appearance of fish

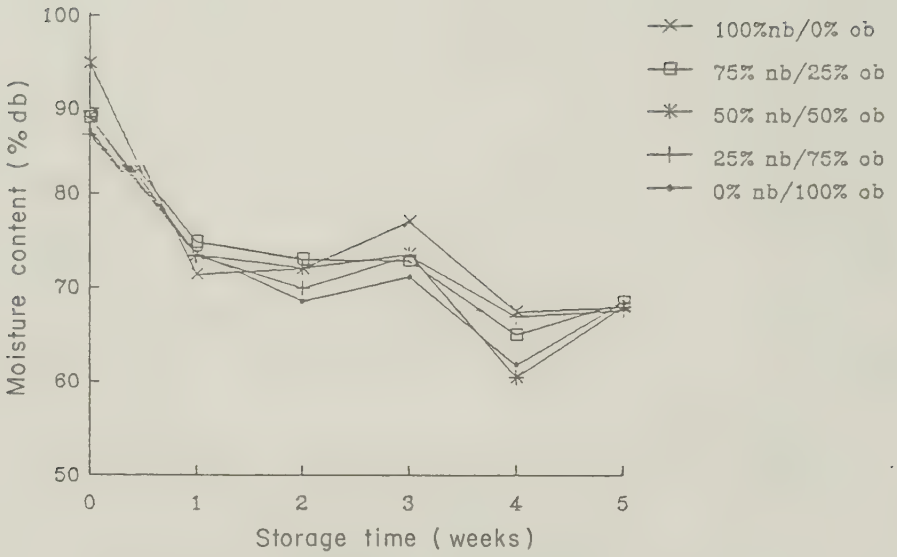


Figure 15 Moisture content of fish during storage

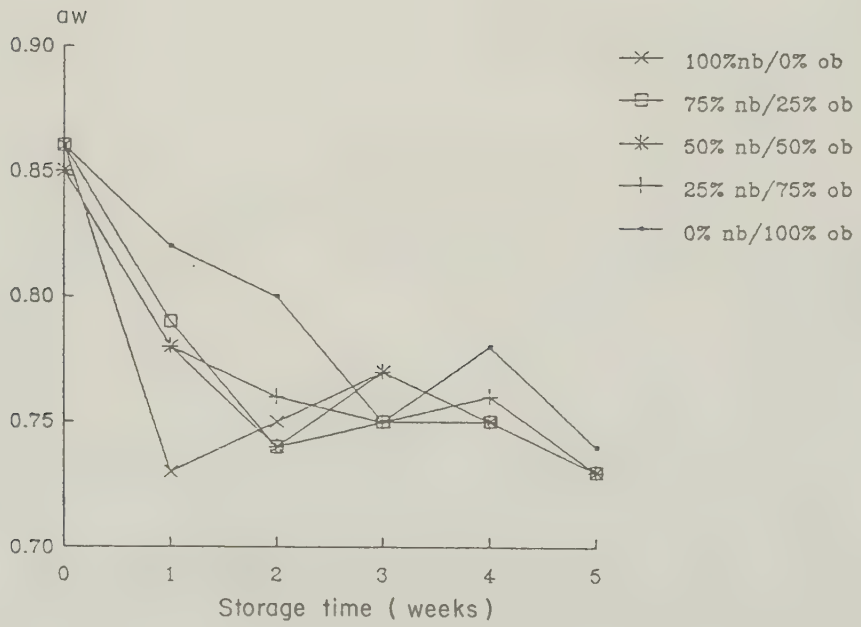


Figure 16 Aw of fish during storage

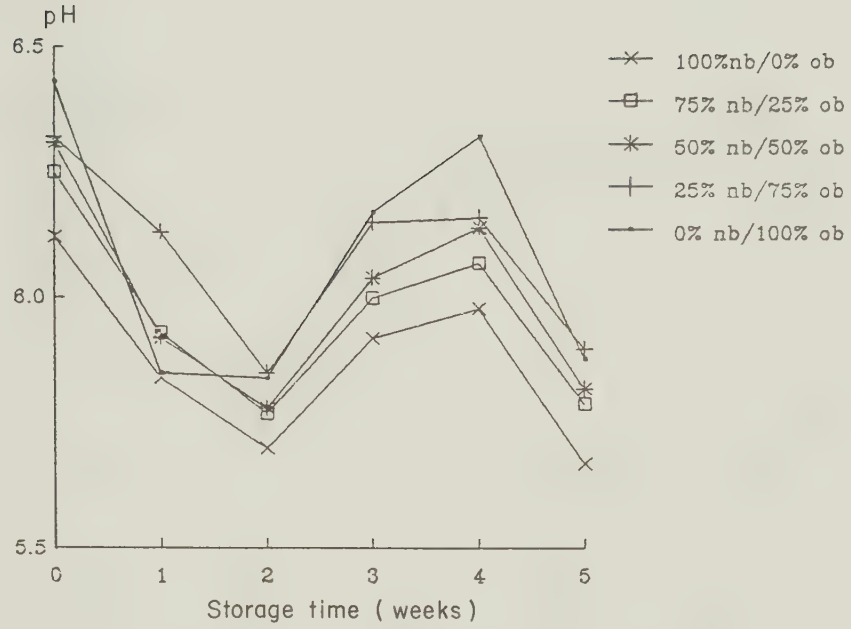


Figure 17 pH of fish during storage

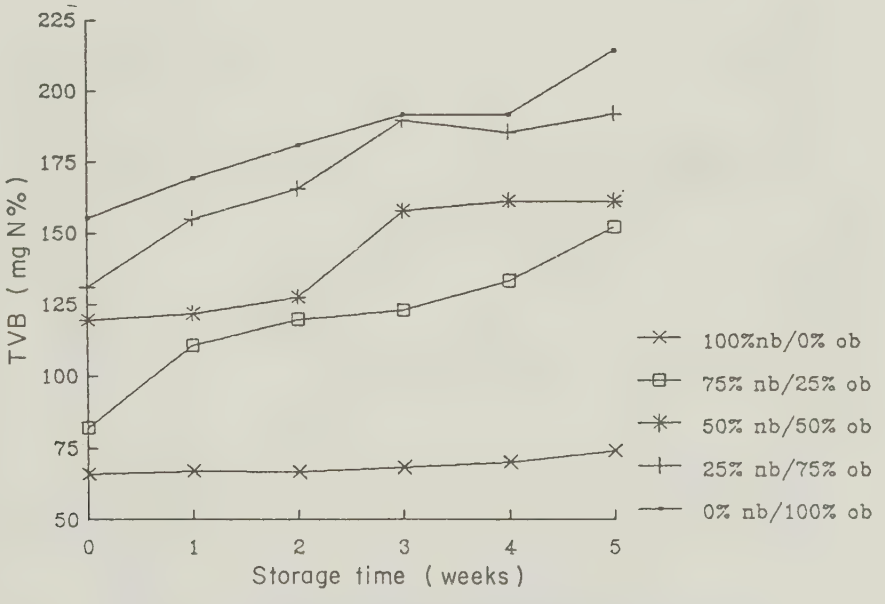


Figure 18 TVB of fish during storage

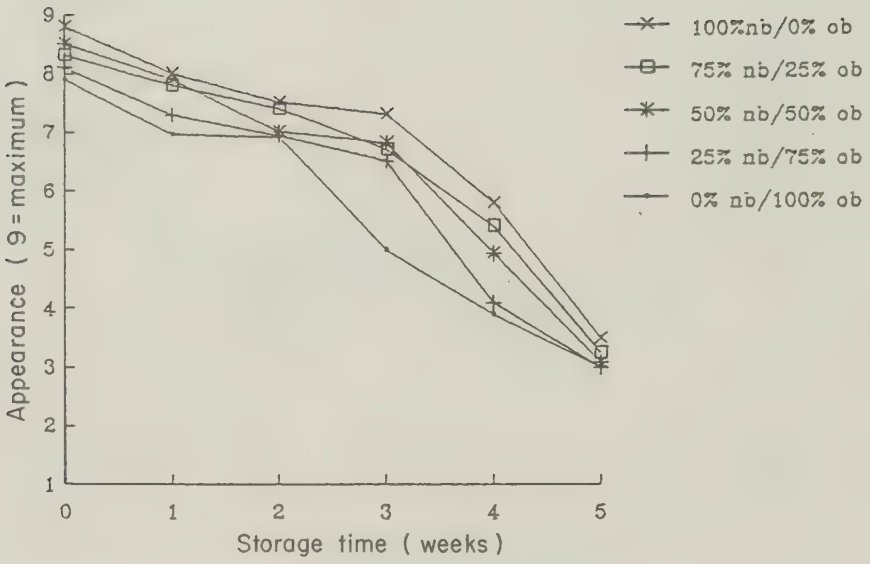


Figure 19 Appearance of fish during storage

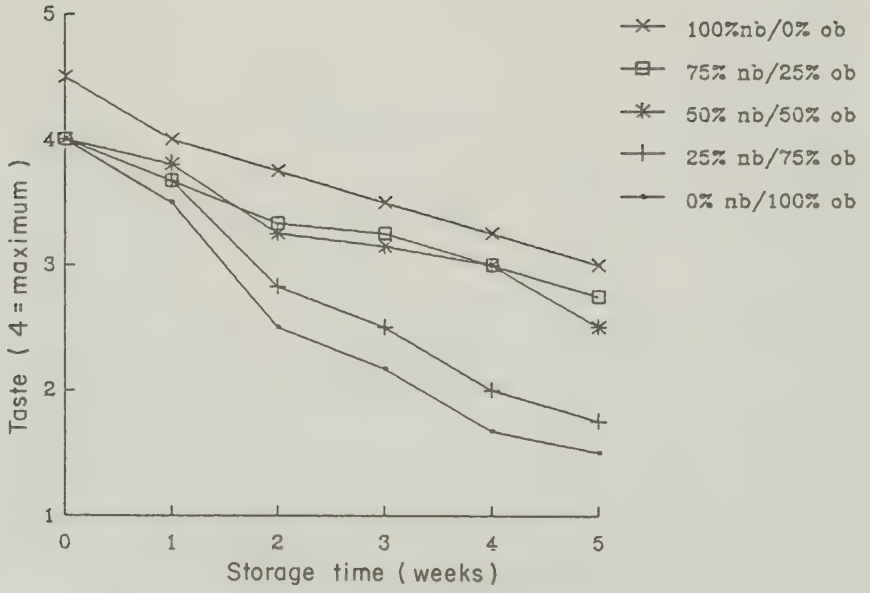


Figure 20 Taste of fish during storage

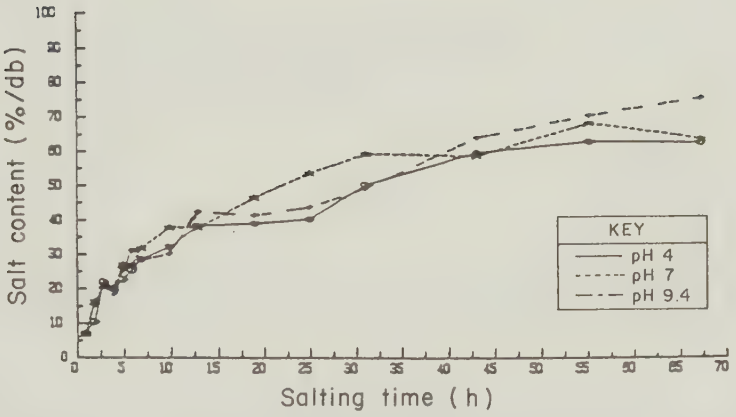


Figure 21 Salt uptake of sardine during salting at 25 °C

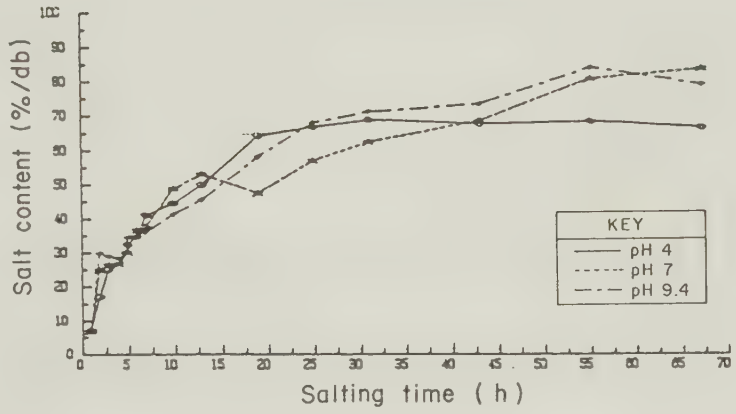


Figure 22 Salt uptake of sardine during salting at 30 °C

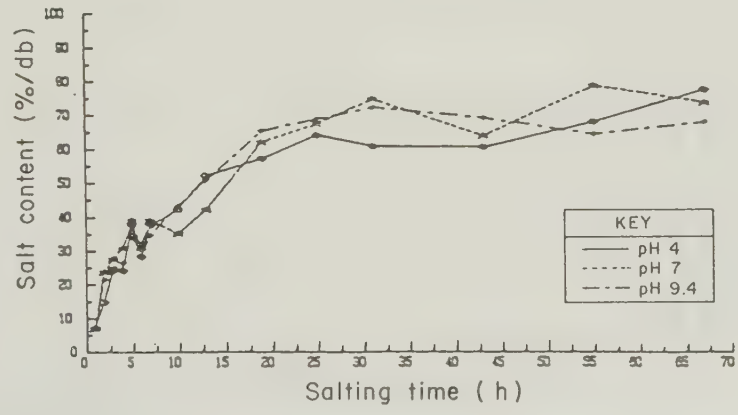


Figure 23 Salt uptake of sardine during salting at 35 °C

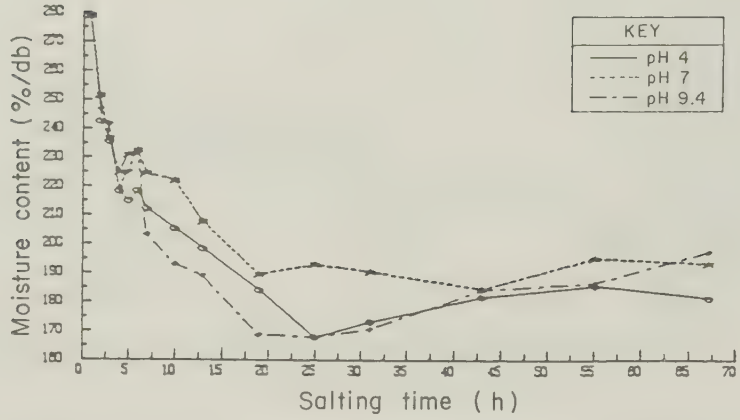


Figure 24 Moisture profile of sardine during salting at 25 °C

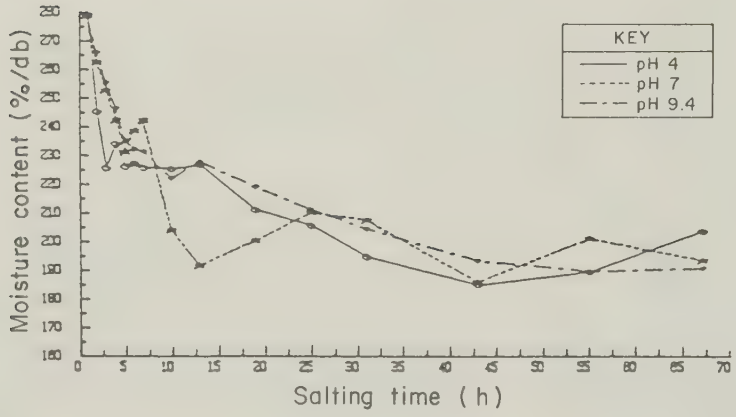


Figure 25 Moisture profile of sardine during salting at 30 °C

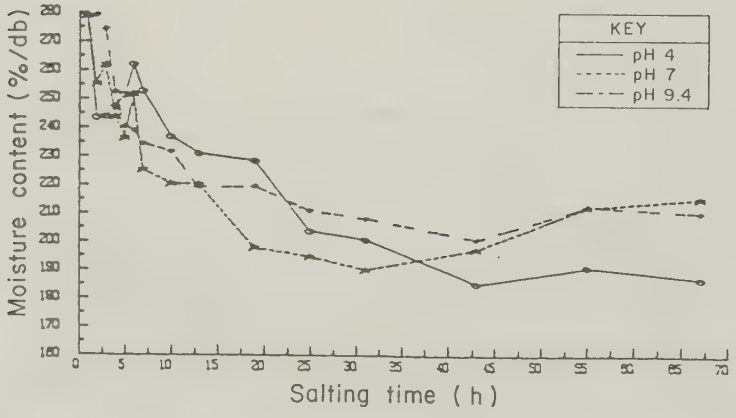


Figure 26 Moisture profile of sardine during salting at 35 °C

THE EFFECT OF BRINE CONCENTRATION ON THE SALTING AND DRYING OF YELLOWTAIL (TRACHURUS MCCULLOCHI)

by

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ABSTRACT

The salting and drying behaviour of yellowtail (Trachurus mccullochi Nichols), a low fat fish caught off the east coast of Australia, was examined. The impact of the process variables on the acceptability of the salted, dried product was also examined.

Wet salting (brine concentrations 15%, 21% and saturated) and three sets of drying conditions (35°C and 50% RH, 45°C and 30% RH and 55°C and 18% RH) were used and assessments were made of salt and moisture content, water activity (a_w) and sensory properties.

Brine concentration during salting was found to have a significant effect on the final moisture content, a_w and the rate of drying, while variations in drying conditions produced no significant effects. The use of higher brine concentrations and drying temperatures resulted in reduced end product quality.

The use of brine with a salt concentration less than saturated could result in a significant reduction in processing time plus improved product quality.

1. INTRODUCTION

In many developing countries where chilling and freezing facilities are often lacking, traditional curing methods are still the main method of preserving fish which cannot be sold fresh.

Indonesia is a major fish producing country whose current annual catch is approaching 2 million tonne, with 34% of the inland catch and 48% of the marine catch being preserved by curing (Buckle et al, 1987). Approximately 70% of the cured fish are salted and dried, while in Malaysia, dried salted fish constitute approximately 12% of the total amount of fish processed (Zain and Yusof, 1983).

In many countries fish is still an important source of low-cost dietary protein and dried salted fish are a significant part of the staple diet. In Indonesia, for example, dried salted fish is one of a group of nine essential food items that are recognized by the Government. To achieve the Government's aim of increasing per capita consumption of fishery products from 12 to over 22 kg/year it is essential that the current high level of post harvest losses are reduced.

The main problems associated with dried fish are the variable, but often low quality of the finished product, its high salt content and the rapid rate of deterioration during distribution and storage. The production of dried fish is still popular in Asia because of the simplicity of the salting and drying process and the relatively small capital investment required.

Although the main attraction of dried fish for consumers is its low price, a major factor preventing its wider acceptance is its strong salty taste. In fact dried fish is losing its appeal as consumer incomes increase and a broader range of food products become available.

The need for the high salt content in traditional dried fish can be attributed to a number of factors which are:

- that sufficient salt must be incorporated into the fish to reduce water activity and hence extend shelf life
- the need to discourage insect attack
- also fish are frequently stored in saturated brine until suitable weather conditions make sun drying possible.

2. RESEARCH PROGRAM

A program was developed to examine the dynamics of the salting and drying process and then seek to refine the process without affecting the factors which make this process popular in Asia. The research was undertaken in the light of the development of a low cost, appropriate technology drier being undertaken in the same laboratories at the University of New South Wales. The research results demonstrate the effect of brine concentration on salting and drying of low-fat fish.

An Australian fish species, yellowtail (Trachurus mccullochi) was used. Its length ranges from 17 to 19 cm and weighs 47 - 65 g. Its proximate composition was measured as: moisture 74%, fat 0.6% and protein 23%.

Fish were divided into 3 groups and immersed in brines of salt concentration 15%, 21% and saturated for 92 h at approximately 23°C. Samples were taken during this period and analysed for salt and moisture content and water activity, and the brining results were plotted against time. The resultant curves clearly show the impact on fish of varying brine concentration.

Figure 1 shows that the rate of salt uptake increases with brine concentration as would be expected and the final value reached depended on the brine concentration.

Figure 2 shows that moisture content decreases at approximately the inverse rate for salt uptake. Moisture content decreases rapidly in the first 10h, the rate depending on the brine concentration. Moisture content stabilised for all 3 treatments after 30 h, as was the case for salt content.

The water activity of the fish brined in each treatment declined rapidly at first and then stabilised after 36 h at a value determined by the concentration of the brine. The water activity for fish in saturated brine stabilised at a value of 0.76 which justifies the traditional practice of storing fish in saturated brine while awaiting suitable drying conditions.

Drying studies were undertaken next to determine the role of brine concentration and the effect of air temperature and humidity on drying rate and product acceptability.

The laboratory drier used for these experiments is depicted in Diagram 1. The unit was designed and constructed at the University of New South Wales and has continuous in-place weighing of the fish as they dry. This makes it possible to plot continuous moisture loss curves on the total sample of fish, thus giving excellent accuracy. Sophisticated control systems also ensure temperature is controlled to an accuracy of 0.5°C and humidity can be controlled to an accuracy of 2% RH during drying.

Fish were again brined in the 3 concentrations mentioned previously and then each group of fish were dried using 3 different sets of conditions until moisture content stabilised. These conditions were:

- 35°C and 50% RH
- 45°C and 30% RH
- 55°C and 18% RH

These temperatures and relative humidities represent conditions based on average ambient conditions for Indonesia taken from that country's weather data.

3. CONCLUSIONS

In order to understand the impact of brine concentration and drying conditions on the final dried product it is best to examine the process as a whole and not just the individual components. This is quite logical when you consider that salt content of the brined fish is the principle determinant of drying rate.

By combining the moisture content curves for salting and drying, it is possible to see the impact of brine concentration on drying rate and the final moisture content of the dried fish.

For the fish processed in saturated brine, temperature and humidity had no impact on drying rate at all. The high salt concentration of the brined fish meant that there was virtually no free water, only bound water in the fish and so, during drying the fish enter straight into the falling rate period without going through the constant rate period of a standard drying curve.

The impact of salt concentration on the drying rate decreases with the concentration value, but the role of salt is always significant and must be understood when changes to a drying process are considered. In fact this is significant information when designing a mechanical drier.

When designing a product the manipulation of water activity is the principle method of maximising the shelf life of that product. Difficulties arise though, when you try to produce a product with a long shelf life that is organoleptically acceptable to consumers. Dried fish is a good example of this situation. Solar drying is notoriously inefficient, consequently processors rely on the extensive salting of fish to lower water activity to a point where a reasonable shelf life is assured. The hidden costs associated with this product are:

1. economic - due to the overuse of salt
2. health - due to the adverse effect of excessive salt intake on the human circulatory system
3. marketing - as a result of the unacceptably salty taste causing consumer resistance.

The results presented in Table 1 indicate that with careful control over the process it is possible to produce dried fish with a low water activity and low salt content.

Each of these products were subjected to sensory evaluation. A panel of 19 assessors who consume dried fish regularly was used. Raw fish were assessed for appearance and texture while cooked fish were assessed for appearance, smell, taste and texture.

The average scores presented in Table 2 for raw and cooked fish samples for each treatment are most enlightening.The average scores for raw fish clearly demonstrate a preference by the panel for fish that have had minimal heat treatment during drying. The panel also showed a preference for fish that had been brined using 15% salt followed by 21% salt. Fish processed in saturated brine was the least preferred, although panelists were prepared to cope with the appearance of salt crystals on the fish in preference to the damage caused by high temperature drying at 55^{o}C.

The scores for cooked fish demonstrate a slightly different set of priorities for tasters. Within the 3 drying treatments for each brine concentration, panelists consistently preferred fish dried at 45^{o}C with fish dried at 55^{o}C being the least preferred. Like the raw fish panelists, a preference was shown for fish brined using low salt concentrations.

Re-examining all the results presented so far the ideal dried fish would be one that is brined using solutions of 15% or 21% salt and dried at 45^{o}C. The salt concentration is low, water activity is low and tasters showed a marked preference for the product. The one problem that prevents the production of this particular product is the reliance by processors on solar drying. The role of an appropriate mechanical drier therefore is most important in encouraging the introduction of a modified salting process in the villages.

The results presented for salted dried fish demonstrate that in conjunction with a mechanical drier considerable savings in time and capital could be achieved over current traditional practices. The processing of small fish by traditional means can take 2 to 5 days depending on weather conditions while a process based on brining in a solution of 15 to 21% salt for 8 to 10 h and drying at 45^{o}C for approximately 12 h would result in a consistently good product in less than 24 h and an improved return on capital investment for traditional processors.

Current research is examining the impact of splitting the yellowtail fish on moisture loss during brining and drying. The work has only recently begun and few results are available but the results presented in Figures 7 & 8 clearly demonstrate the effect of splitting fish.

Fish were brined in solutions of 5%, 15% and saturated salt and then dried at 45^{o}C and 30% RH. Except for fish brined in 5% salt, splitting had little impact on moisture loss. Splitting was in fact detrimental to moisture loss for fish brined in 5% salt. The moisture loss during drying is quite different for split and whole fish. Split fish lose moisture at a more rapid rate than is the case for whole fish. This result was expected due to the much higher surface area to volume ratio of split fish and the lack of skin and scales which would otherwise impede moisture loss. Using split fish, it would be possible to brine and dry fish in 10 to 12 h using a mechanical drier.

Ultimately this type of research will lead to greater efficiencies and improved return on capital investment for traditional processors, as well as benefiting consumers.

4. REFERENCES

Buckle, K.A. et al. 1988 Studies on the stability of dried salted fish. In Seow, C.C., ed. Preservation by moisture control. London, Elsevier Applied Science, p 103-115.

Zain, A.M. and Yusof, S. 1983 Salt and moisture contents of Malaysian dried fish. James, D., ed. Proceedings of the Workshop on the Production and Storage of Dried Fish. FAO Fish.Rep., No. 279.

Figure 1. Salt uptake of yellowtail during salting at ambient temperature

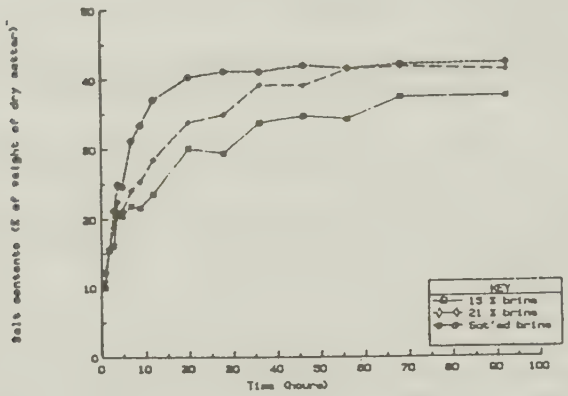


Figure 2. Moisture profile of yellowtail during salting at ambient temperature

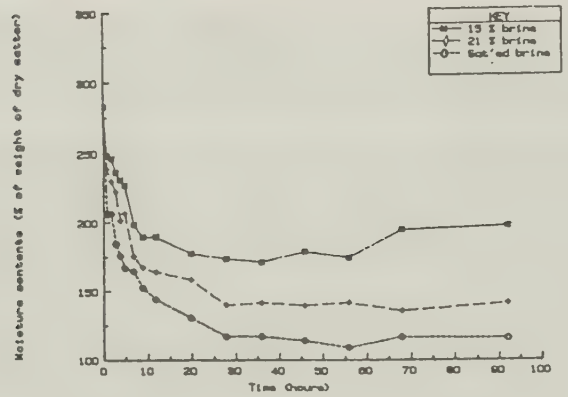


Figure 3. Water activity profile of yellowtail during salting at ambient temperature

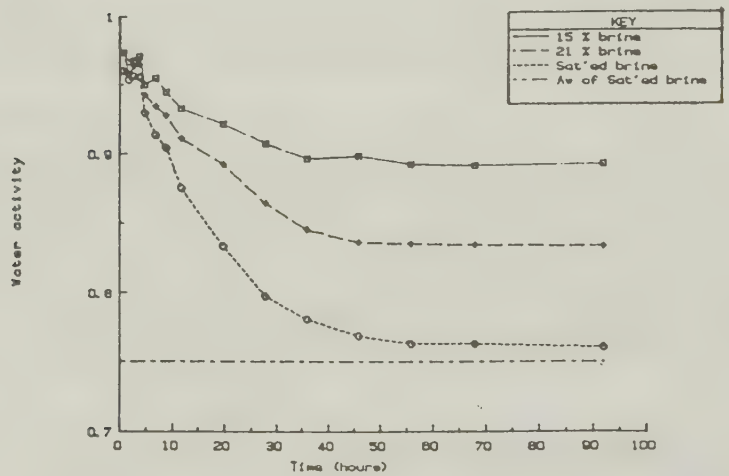


Figure 4. Fish brined in 15% salt solution

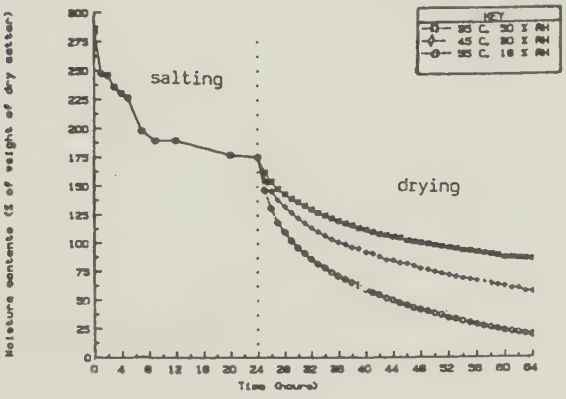


Figure 5. Fish brined in 21% salt solution

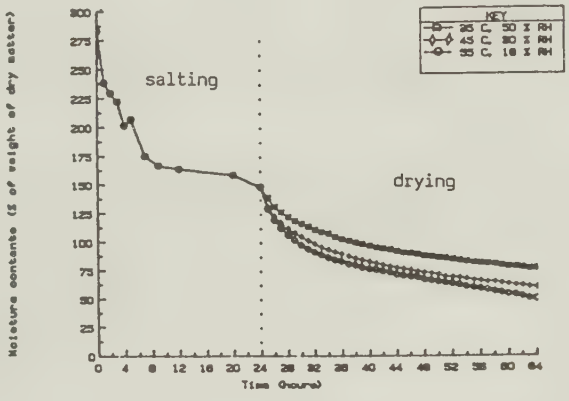
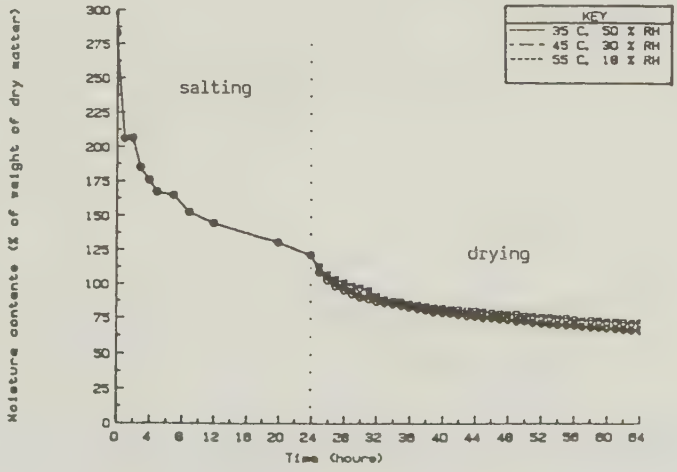


Figure 6. Fish brined in saturated salt solution



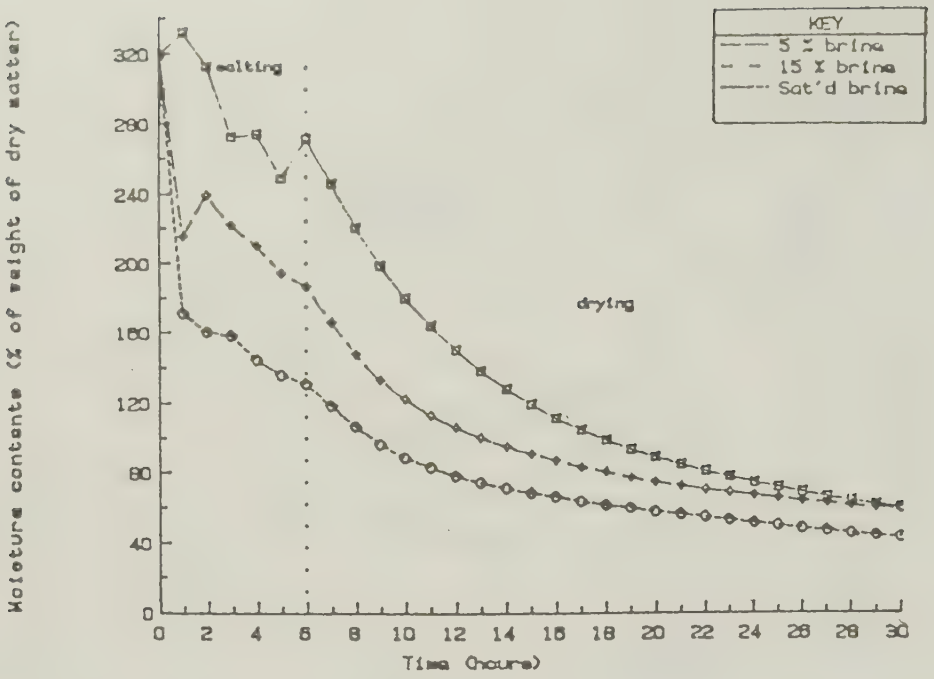


Figure 7. Salting tne drying curves of split yellowtail which was brined in different brine concentrations and dried in 45C. 30% RH and 2 m/s air velocity

Figure 8. Salting and drying curves of whole yellowtail which was brined in different brine concentrations and dried in 45 C, 30 % RH and 2m/s air velocity

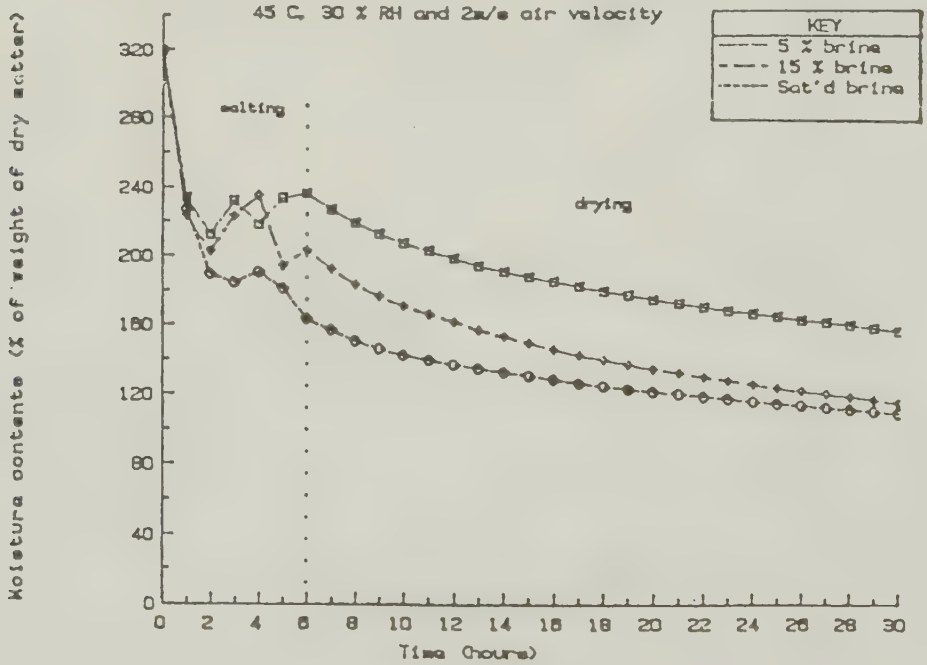


Figure 8. Salting and drying curves of whole yellowtail which was brined in different brine concentrations and dried in 45 C. 30% RH and 2 m/s air velocity

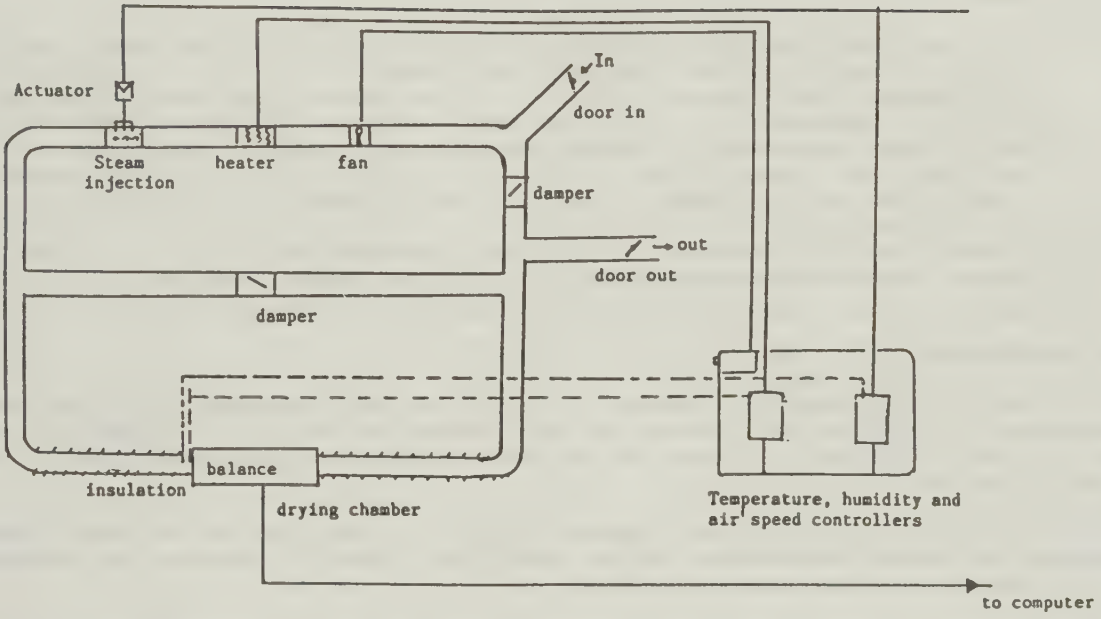


Diagram 1. Schematic diagram of the fish drying system

DEVELOPMENT OF A RICE HUSK-FIRED FURNACE AND ITS APPLICATION TO FISH DRYING

by

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ABSTRACT

A project jointly funded by the Australian Centre for International Agricultural Research (ACIAR) and the Government of Indonesia (GOI) commenced in 1984 and aimed at reducing the losses associated with dried fish production. Solar drying was one aspect of production that was quickly identified as a significant source of loss and where improvements were required.

Solar drying is vulnerable to inclement weather; a continuing problem is to find a drying system that is of a level of technology appropriate to the end user and satisfies a number of critical economic criteria. To date no drier design has been commercially successful in South Asia.

This paper describes the development of an appropriate technology furnace which uses rice husks as an energy source. Flue temperatures in excess of 800^{o}C have been achieved for prolonged periods. A drying cabinet that would utilise the high flue temperatures has been designed, constructed and initial tests have demonstrated its potential. Neither the furnace nor drying chamber requires any additional power source or fans to move the air in the chamber. Initial costings appear to satisfy the economic criteria to ensure commercial viability,.

Further research is required to determine the acceptability of other agricultural wastes as fuel and to expand the range of products that can be dried.

1. INTRODUCTION

A paper was presented at the Sixth Session of the IPFC Working Party in 1984 which gave an overview of a new fisheries project, which commenced in 1984 and was jointly funded by the Australian and Indonesian governments.Its aim was to reduce the losses associated with dried fish production and marketing.

The project has now concluded, and some, but not all the aims were achieved. Researchers in Indonesia successfully examined and defined traditional dried fish practices and commenced studies on improvements to current packaging systems.

In Australia, research at the University of New South Wales examined the salting and drying dynamics of dried fish production, as well as possible alternatives to traditional solar drying.

Solar drying has long been recognised as a significant cause of loss in dried fish, mainly as a result of the lack of control over the final moisture content of the product and prolonged drying times. The unpredictable nature of weather means that under ideal conditions small fish can be dried in one day but when inclement weather predominates, drying may take 3 to 5 days. There have been many attempts to develop a suitable alternative to solar drying and to date there have been no commercially successful, low technology driers developed in Asia. A suitable drying system must be of a level of technology appropriate to the end user and satisfy a number of critical economic criteria.

2. PROJECT DESCRIPTION

As mentioned earlier the project examined alternative drying systems. Our approach has been to study traditional fish processing and discuss at length with processors the essential or desirable features of any drier that would find alternate use to solar drying. Predictably the points featured in Figure 1 are essentially those which apply to solar drying. Acceptance of a new drying system by village processors required our acceptance of the design constraints placed upon us by this list.

An obvious way to keep fuel costs low is to make use of agricultural wastes. In Indonesia a large quantity of this material is available, with a potential energy value equivalent to 16×10^{6} t

of coal per annum. In 1986 it was estimated that Thailand produced more than 66×10^6 t of agricultural waste with an average energy value of 12.5 megajoules/kg giving a total potential energy source of 66×10^6 gigajoules (Bhattacharya, 1986). This represents about 6 times the annual fuelwood consumption of Thailand. This alternative energy source is significant when you consider that in Thailand for example over 90% of households rely on firewood as the primary source of energy, while also in Thailand between 1951 and 1977 the area under forest as a proportion of total land area has declined from 53% to 25% (Phichitvorapanich, 1987).

Agricultural wastes are often difficult to burn efficiently, requiring an intimate knowledge of the fuel together with the development of novel technology to cope with the combustion difficulties of individual fuels. After some research rice husk was chosen as the target fuel source for this project mainly because of their ready availability, in large quantities, to the coastal fishing villages of Java, Indonesia.

In 1978, more than 60 million tonne of rice husk was produced as a by-product worldwide and its disposal represented a serious pollution problem (Beagle, 1978). Owing to their abrasive character, poor nutritive value, high resistance to degradation, very low bulk density and high ash content, the normal method of disposal is to burn the husk.

Rice husk do have some advantages though over other agricultural wastes which include:

1. low moisture content as it leaves the mill

2. easily compressed into bales eliminating the problem of transporting a low bulk density material

3. they are often more easily ignited

4. and because of the widespread cultivation of rice, husks are readily available year round in Asia.

Rice husks have about half the energy value of coal and they constitute some 20% of the annual Indonesian rice crop of 38×10^6 t. Figure 2 shows that rice husks could provide a source of energy equivalent to 3.6×10^6 t of coal. Currently these rice husks are used as a fertiliser or occasionally burnt and the ash used as a domestic abrasive cleaner.

The proximate analysis of rice husk is presented in Table 1. The volatile matter is that portion of the husk which is driven off in a gas or vapour form when the husks are subjected to heating and consists of hydrocarbons and other gases. The fixed carbon represents the combustible residue left after the volatiles have been driven off. The material left after complete combustion of the husk is ash which is nearly 100% silica.

With an approximate energy value of 13 megajoules/kg, 1 kg of rice husk could produce 2.5 kg of steam. It has been calculated that a rice mill with a capacity of 1 tonne per hour could produce sufficient steam from the waste husk to run the mill and still leave 20% surplus for drying and parboiling, (Phichitvorapanich, 1987).

When considering combustion of rice husk, the physical shape of the rice husk particles and the structure of the fuel bed are more important than moisture content, volatile matter, ash content and even energy content. The design of a suitable furnace is therefore the most critical component of the project work.

Basically there are two methods of burning husk.

1. Combustion - that is the rapid chemical combination of oxygen with the combustible elements of the fuel.

2. Gasification - when fuel is burnt with limited oxygen so that combustion is not complete. The by-products of gasification include methane, carbon monoxide and hydrogen - these are referred to as producer gas.

The gasification of rice husk is a more attractive proposition than combustion because it is possible to burn the producer gas like propane or LPG without smoke or particulate matter in the flue gas. Gasification is the result of a chemical process called pyrolysis, which is the thermal decomposition of a substance resulting in the liberation of the constituent components of that substance. These components are usually extremely volatile.

There are many designs for biomass gasifiers, (Phichitvorapanich, 1987) and are given as follows:

1. The updraught type,where fuel is fed in at the top and air is forced through the grate at the bottom while the producer gas is collected at the top.

2. The downdraught type,where again fuel is fed in from the top, but air is introduced through horizontal nozzles. Producer gas is collected at the bottom edge of the unit.

3. The crossdraught type in which air is introduced in one side of the bed, burns and producer gas emerges at the opposite side.

4. The fluidised bed gasifier which has many advantages over the previous systems but is very complex and so too expensive for low level processors.

Up till now gasifiers were primarily designed to access the producer gas for use in modified internal combustion engines or through complex burners. These units did not fulfill the criteria presented in Figure 1 which lists the desirable features for a mechanical drier.

3. RESULTS

During the course of the project a novel rice husk furnace (Figure 3) that satisfies our design criteria has been designed, constructed and tested. The furnace is easily constructed of readily available materials, has no moving parts and uses rice husk as a fuel.

The unit is based on a used 200 L drum equipped with a central perforated tube and a perforated base plate. It could be constructed in about 6 h and has been costed at less than US$20 when constructed in Indonesia.

Although the design is rather simple, determining and quantifying the design parameters has proved a lengthy and on-going exercise. These parameters were quickly identified as follows:

1. Centre tube specifications: its diameter, the ratio of holes to surface area (or percent open space) and the diameter of the holes.

2. Base plate: the critical factor is the percent open space and the diameter of the holes in the base.

3. Drum : there exist a complex relationship between the diameter and height of the drum and the diameter of the centre tube.

4. Start-up procedure : a short period of high temperature input is required to start the furnace.

Each of these performance variables interact with each other to produce a clean, high temperature flue gas, so altering one variable only by a small amount can have a significant impact on the performance of the furnace.

An outline of the operation of the furnace would be as follows:

1. Approximately 27 kg of husk is added to the drum.

2. Intense heat is applied to the base of the furnace.

3. Within 15 minutes a zone of oxidative combustion is initiated in the base of the husk bed. At this stage the heat source can be removed.

4. As the combustion zone moves up into the bed and oxygen becomes limited a pyrolysis zone develops.

5. The pyrolysis zone gradually moves up through the bed and is now self-sustaining.

6. Volatiles released by pyrolysis pass into the centre tube where they spontaneously ignite. The heat passes out the flue, while some of the heat passes back into the fresh husk above the pyrolysis zone and initiates a new zone of pyrolysis. This process continues until the zone reaches the top of the drum.

During the two years that development of the furnace has been in progress many experiments have been done first by filling the drum only once and allowing it to burn out, each time varying one parameter. Later, as the design was close to completion attempts were made to extend the period of heat output, with the aim of running the furnace continually. The furnace was extensively instrumented to enable monitoring of conditions in the fuel bed and the output in the flue. Fifteen thermocouples were installed in the furnace in 5 rows of 3. This allowed continuous monitoring of

the temperature profile throughout the fuel bed. Also flue gas temperature was recorded as well as oxygen and carbon monoxide levels in the gas. The complete furnace was placed on a load cell so that weight loss could be recorded and weight loss is directly related to the combustion of carbon. The rate of carbon combustion gives a good indication of operational efficiency.

Flue temperatures ranging from 800 to 1000°C have been achieved for prolonged periods. The energy output has been calculated at 12-14 kW for sustained periods with peak outputs of 20kW. This output represents the efficient extraction of energy from the rice husks which is estimated at 80%. By comparison a car engine is about 16% efficient.

Figure 4 shows the flue temperatures for two recent experiments, one of which was sustained for 6h before being allowed to burn out. To run the furnace for periods in excess of 4h requires the regular removal of ash from the base and the addition of fresh husk at the top of the furnace is required. In this way the pyrolysis zone can be maintained at the midpoint of the furnace resulting in maximum output. The design parameters have been quantified and continuous operation is now possible, but there still exists some operational problems with the furnace.

The start-up procedure is another critical stage of furnace operation. The time for the furnace to come up to operating temperature should be as short as possible, firstly for efficient operation and secondly the furnace does produce considerable smoke before pyrolysis begins in the fuel bed.

Many methods of getting heat into the base of the fuel bed have been tried. A gas burner and a wood fire have been tested, but work on a duplicate furnace in Jakarta by my colleagues has developed a rapid, appropriate method of attaining operating temperature of 800°C in the flue. Originally times in excess of 1h were needed to reach 800°C but as the base design was modified times came down to about 40 minutes using a gas burner. This time was reduced further by the work in Jakarta, using charcoal soaked with kerosene so that now the furnace is operational within 30 minutes.

The removal of ash which is at a very high temperature represents a significant problem. The difficulty arises in designing a removal system that is cheap and easy to construct while allowing the uniform removal of ash from the full cross-section of the drum. One other area requiring further research is the material that could be used to construct the furnace centre tube that would maximise the operational life of the unit. Ceramics are one possibility, which have a long history of use for stove construction in Asia and also have the advantage of being cheap and easy to produce.

The use of this furnace in the drying of fish represents a separate problem while still being guided by the original criteria for the furnace. There are many possible means of using the hot flue gases for drying fish, but most are unacceptable when capital and running costs are taken into account.

Before designing a drying chamber, experiments were done at the University of New South Wales to determine the drying requirements of salted fish.

There are three conditions within a drier that can be controlled and effect drying rate:

- Air temperature
- Air relative humidity
- Air velocity

In Asia, ideally it would be best to dehumidify air before it enters the drying chamber to dry a product efficiently, but the equipment necessary to do this is expensive and complex. Therefore because we were designing a low technology drier for village use it was decided to rely on air temperature control to manipulate relative humidity.

After considerable research the conditions to dry salted fish as efficiently as possible while also producing a product of acceptable quality were found to be:

- air temperature - 40 to 45°C
- relative humidity - that level which corresponds to tropical ambient air heated to the above temperature range
- air velocities - 0.25 to 1 m/s

The drying curves produced by this research showed that salted fish do not follow a standard drying curve, that is they do not go through the two phases of drying which are:

1. the constant rate period

2. the falling rate period

In fish that have been salted, nearly all the water in the fish is chemically bound and so when dried, fish go directly into the falling rate period. During this period air velocity is not a critical factor, unlike air temperature and humidity.

Because air velocity was found not to be a major requirement of fish drying it was possible to omit mechanical methods of moving air when designing the drier. In fact a system of moving low volumes of air called a natural draught drier was adopted, thus preserving the drier's low level of technology.

Figure 5 shows an outline of the drying chamber in relation to the furnace. Air is encouraged to move through the drying chamber and thereby flush out the moisture as it evaporates from the fish by relying on the temperature differential between the flue above the fish and the ambient temperature. A difference of approximately 300 to 400°C would give a velocity of 0.5 to 1 m/s in a chamber 1 m^2.

Essentially, with this design, the hot flue gas pass through the space formed through the floor and wall by a piece of corrugated metal which acts as a heat exchanger or radiator. The external walls should be made of an insulated material of low thermal mass so that the walls do not deprive the chamber of heat.

The major task in drier design is matching the output of the furnace to the drying cabinet size and therefore drying capacity. A floor area of 1 m^2 was selected as a fundamental measurement because a 1 m^2 tray which would hold up to 10 kg of product can be carried, loaded and unloaded by one person. The chamber depicted in Figure 4 is 1 m high, this size was based on calculations prior to constructing and testing the unit.

Initial results from this chamber were disappointing. It was not possible to simultaneously maintain a drying temperature of 45°C in the chamber as well as a flue temperature of 300 to 400°C that is required to induce a natural draught.

This failure is due to the choice of bricks for the walls, which have a large thermal mass and the overall size of the drying chamber in relation to furnace output.

4. CONCLUSIONS

Considerable work is still necessary to finalise the chamber design, but there must be extreme flexibility when seeking solutions. For example, it may not be necessary to change the chamber design radically if it is already suitable for village use, but by connecting two furnaces, which have already been shown to be very cheap, sufficient energy may be available to effectively dry the fish.

After stating previously that for all the research, no commercial appropriate technology drier had been adopted in Asia as a replacement for solar drying, the obvious question is why should this particular furnace and drier be successful where others have failed?

In recent years a greater understanding of the difficulties of transferring technology from one community to another has evolved. As part of this project the essential elements that would ensure the successful incorporation of an appropriate drying system into village level Indonesia have been defined. These necessary elements have 2 components as shown in Figure 6.

Ideally the appropriate technical solutions to the problems of a community should be developed in that community. As that is not currently possible in Indonesia, the close collaboration between Australian and Indonesian scientists is the next best way of ensuring the successful integration of a drying system into the traditional process.

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Table 1

Proximate analysis of rice husk

	% (DB)
Volatile matter	55 - 60
Fixed carbon	13 - 17
Ash	16 - 23

Figure 1. Desirable features of a dryer

1. No fuel cost
2. No moving parts
3. Low capital cost
4. Low running and maintenance cost
5. Local materials for construction
6. Constructed, repaired by local tradesmen
7. Low additional cost (e.g. labour) over sun drying.

Figure 2. Indonesian agro waste as an energy source

Waste	Coal Equiv. (Mill. T/y)
Rice straw	0.9
Rice husks	3.6
Wood waste	1.2
Saw dust	0.9
Coconut husk	6.8
Baggase	2.3
TOTAL	15.7

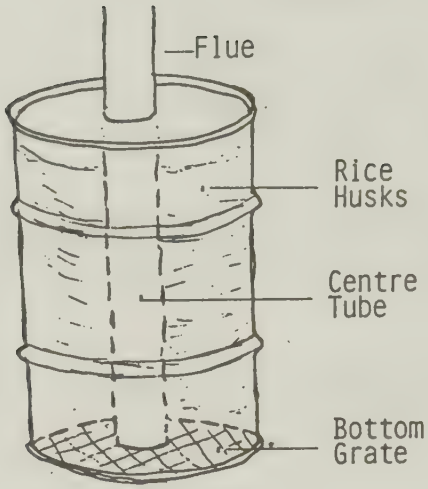
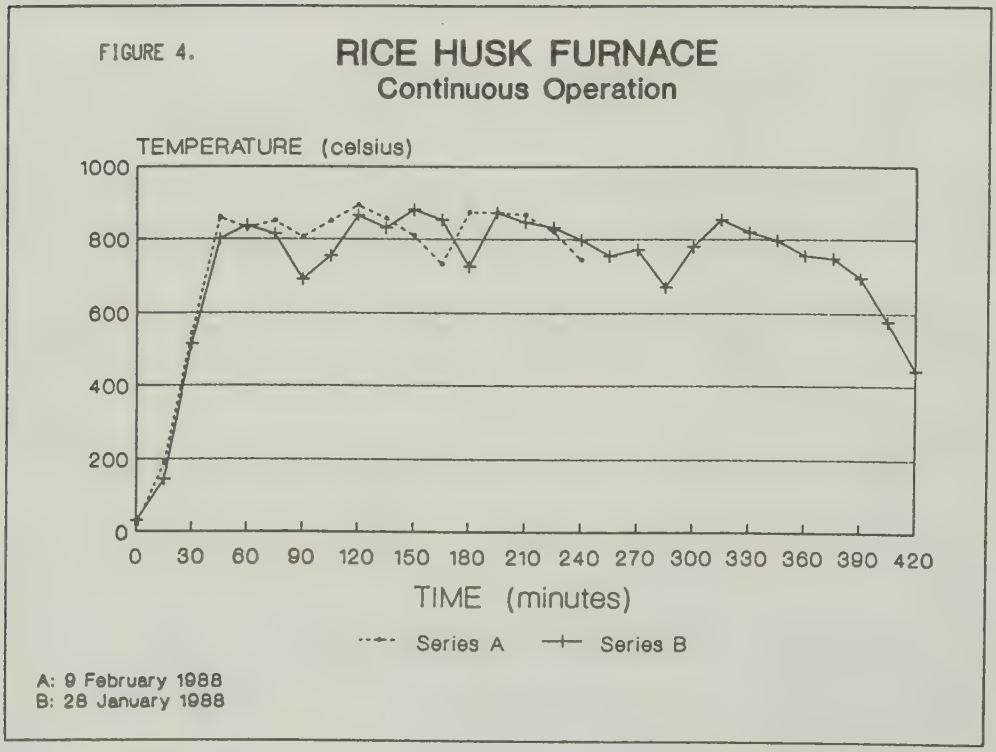


Figure 3 Rice Husk Furnace
Capacity: 27 kg
Peak Energy: 20 KW
Flue: 900-1000°C
Period: 4-5 hours



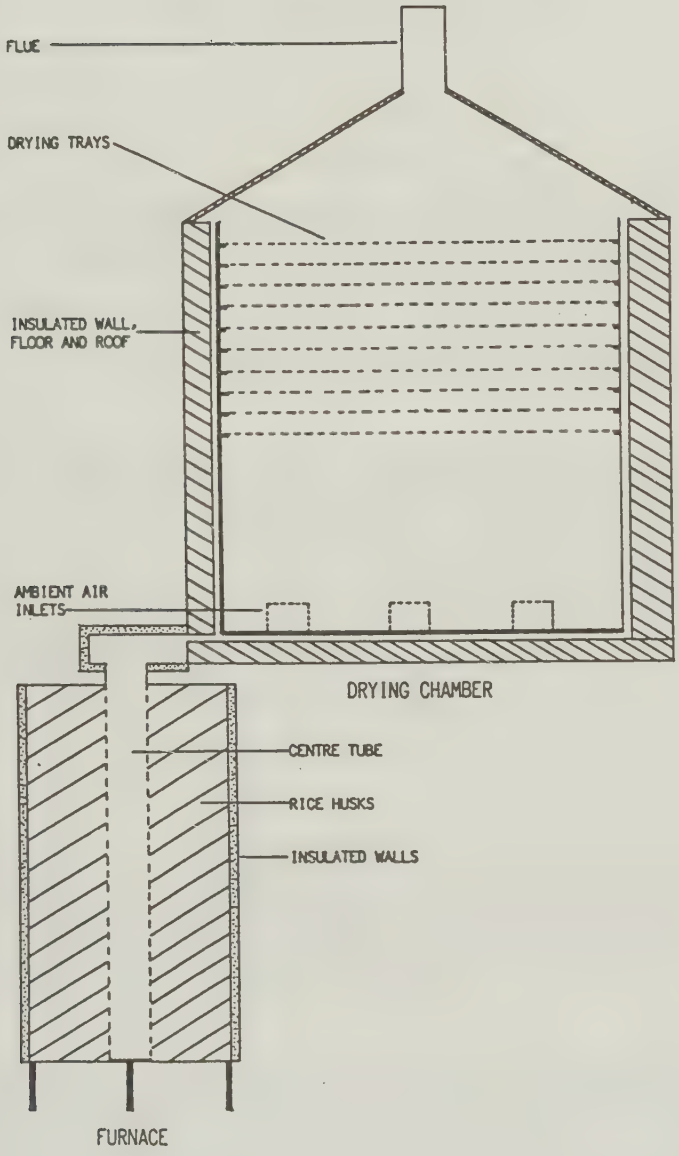


Figure 5. Schematic of rice husk furnace drying chamber

PREVENTION OF INSECT INFESTATION AND LOSSES OF SALTED-DRIED FISH IN INDONESIA BY TREATMENT WITH AN INSECTICIDE APPROVED FOR USE ON FISH

by

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ABSTRACT

Pirimiphos-methyl is an organophosphorus insecticide which has been cleared by FAO/WHO for use on fish and has recently obtained provisional registration (under the brand name Minawet) for use on salted-dried fish in Indonesia.

A series of trials, evaluating the effectiveness of pirimiphos-methyl in preventing insect infestation of salted-dried fish during processing and storage, have been carried out in five provinces of Indonesia, as part of a training project funded by the Government of Indonesia and the Overseas Development Administration.

These trials have consistently demonstrated that pirimiphos-methyl, when applied as a 0.03% a.i. dip, protects salted-dried fish against blowfly infestation during processing and further insect infestation during storage.

Fisheries quality control staff from twelve provinces in Indonesia are receiving training in the safe use of pirimiphos-methyl, as part of a more general training program in post-harvest loss assessment and reduction. This training will be extended to traditional fish processors by the Indonesian Directorate-General of Fisheries and the insecticide manufacturer, ICI, to ensure that pirimiphos-methyl is adopted as a safe and effective alternative to the unapproved use of household insecticide.

1. INTRODUCTION

Indonesia is a major producer of fish with a total production of 2.067 million tonnes in 1985. Marine production accounted for about 76% of the total. Fish is a nutritionally important commodity in Indonesia and provides approximately 70% of the per capita, dietary, protein intake. Curing, in particular salting and drying, is a major, and in some areas, the only method of fish preservation available to the fish processor. Approximately 40% of the marine production was processed by traditional methods in 1985, mainly by salting and drying and it is predicted that traditionally processed fish will continue to be a nutritionally and economically important commodity in the foreseeable future.

Field and laboratory investigations carried out by the School of Food, Fisheries and Maritime Studies, Humberside College of Higher Education, and The National Centre for Quality Control and Fishery Processing Development in Indonesia, have revealed that cured fish losses, both financial and of edible flesh, constitute a major problem for the fish processing industry in Indonesia. Two of the main causes of losses were found to be blowfly infestation during processing and beetle infestation during storage. Some cured fish processors and retailers have consequently resorted to treating their fish with unapproved, household insecticides, such as Baygon and Startox, in order to reduce insect infestation. This illegal practice is particularly prevalent in Java and is recognised as being both undesirable and hazardous by the Directorate-General of Fisheries in Indonesia.

Field trials to evaluate alternative, non-hazardous methods of preventing insect infestation were carried out in West Java between 1983 and 1986 (Esser et al., 1986). A major recommendation resulting from this work was that the FAO/WHO approved insecticide, pirimiphos-methyl, should be registered for use on cured fish in Indonesia, and that its introduction to fish processors would be

best achieved under the auspices of the current Overseas Development Administration/Government of Indonesia funded project "Post-harvest losses of fish in Indonesia".

One of the major aims of this project is to provide staff of the Fish Quality Control Laboratories, at several centres in Indonesia, with training in loss assessment and safe, loss reduction techniques. To date, training programs have been undertaken by Department of Fisheries staff in East Java, North Sumatra, Central Java, West Kalimantan and South Sumatra. Similar training programs are to be carried out in Lampung, West Sumatra, Sulawesi and West Java before the project is completed in 1989.

The training program, conducted in each province, consists of lectures and seminars on the assessment and reduction of losses of traditionally processed fish, together with practical training in survey techniques and field trials. A major emphasis of the training program has been to provide training in the use and evaluation of the FAO/WHO approved insecticide, pirimiphos-methyl, in reducing insect infestation and losses of cured fish during processing and storage. Pirimiphos-methyl has recently received provisional registration for use on fish by the Indonesian authorities and is to be marketed in Indonesia as Minawet 250 EC.

The pirimiphos-methyl evaluation trials, carried out as part of the training programs given in East Java, Central Java, North Sumatra, South Sumatra and West Kalimantan, have consistently demonstrated that pirimiphos-methyl, applied as a 0.03% a.i. dip, protects fish against blowfly infestation during processing and against Dermestes and Piophila infestation during storage. This paper describes field trials carried out in three provinces of Indonesia as part of the loss assessment/reduction training program. The processing trials detailed here were conducted in West Kalimantan and the storage trials in North Sumatra and Central Java.

2. BLOWFLY INFESTATION DURING PROCESSING TRIALS CONDUCTED IN PONTIANAK, WEST KALIMANTAN

2.1 Materials and Methods

Processing trials to demonstrate the use of pirimiphos-methyl in protecting fish from blowfly infestation during drying were carried out at a commercial fish processing site in Pontianak, West Kalimantan. Three trials, in each case using marine catfish, Arius sp., were performed. Trial A was to demonstrate to the staff of the West Kalimantan Department of Fisheries, methods of assessing and reducing blowfly infestation and damage during processing. Trial B was conducted by the Department of Fisheries staff. Trial C was carried out jointly by the staff of the Department of Fisheries and Humberside College, to investigate the effect of infestation of varying the pirimiphos-methyl dipping time. The trials took place in November, during the wet season, when conditions are most favourable for blowfly infestation.

The fish used in Trials A and C were caught by the processor's fishing fleet and were stored in ice on the boat. The fish in trial B was purchased locally by the processor.

On arrival at the processor's premises, the fish were individually labelled, decapitated, split adjacent to the backbone, eviscerated and the muscle was cut several times longitudinally. The fish were weighed and placed (skin up), with alternate layers of salt, in a plastic barrel which was then covered with a plastic sheet. With the exception of labelling and weighing the fish, this procedure was as normally practiced by the processor. The fish were left in salt overnight. The following day the fish were inspected for larval infestation. After being washed by the processor, as was his normal practice, the fish were re-weighed to determine the weight loss during salting. In each trial the fish were ranked in order of pre-salting weight and divided into groups of similar weight range.

Trial A fish were divided into two groups of 22. The fish of one group, designated Control, were individually dipped for 15 seconds in water. Fish belonging to the second group, designated Minawet, were dipped for 15 seconds in 0.03% a.i. pirimiphos-methyl (water based emulsion). In both Control and Minawet treatments, 11 fish were dipped per 6 L of water or insecticide.

Trial B was divided into two groups of 20 fish. One group, designated Control, was dipped for 15 seconds in water. The second group, designated Minawet, was dipped for 15 seconds in 0.03% pirimiphos-methyl emulsion. In both Control and pirimiphos-methyl treatments, 10 fish were dipped per 5 L volume of water or insecticide.

Trial C was divided into four groups of 10 fish. The first group, designated Control, was dipped for 15 seconds in water. The second group of fish, designated Minawet 15 seconds, was dipped in 0.03% pirimiphos-methyl for fifteen seconds. The third group, designated 10 seconds, was dipped in 0.03% pirimiphos-methyl for ten seconds. The fourth group, designated Minawet 5 seconds, was dipped in 0.03% pirimiphos-methyl for five seconds. In all treatments 10 fish were dipped per 6 L.

In each trial the fish were put on drying trays which were placed randomly in the sun to dry. Different treatments within each trial were allocated separate drying trays. Each night (and every time it rained) the drying trays were stacked up in separate piles for each treatment of each trial and covered with plastic sheets. Each morning when the fish were uncovered and every evening before they were covered, the fish were individually examined for the presence of freshly laid blowfly egg batches and for larval infestation. New egg batches were tagged and recorded. Larval infestation was graded as follows:

Code	Observation
0	No larvae present
L	Occasional larvae
M	10-50 scattered larvae
H	Numerous larvae, feeding packs

During the first two days of drying, blowfly counts were carried out every 15 minutes, i.e. the fish were inspected to determine the number of Chrysomya megacephala Fabricius (Diptera: Calliphoridae) present on the fish of each treatment.

The fish were left to dry until the processor judged the process to be complete. After drying the fish were weighed and inspected for larval infestation and damage. The damage was recorded as follows:

Code	Observation
0	No damage
L	Light damage, <1% of fish surface damaged
M	Medium damage, 1-10% damaged
H	Heavy damage, >10% damaged

2.2 Results and Discussion

Figures 1 to 3 show the blowfly counts, i.e. the number of C. megacephala present on the fish of each treatment, at 15 minute intervals, during the first two days of drying. The graphs also show the variation in temperature and humidity during drying. Figures 4 to 6 show the number of egg batches laid on the fish of treatment for each day of drying. Figures 7 to 9 show the degree of larval infestation on the fish of each treatment, in the morning and evening for each day of drying. Both C. megacephala and Lucilia cuprina Wid. (Diptera: Calliphoridae) were observed on the fish during drying. C. megacephala was present in far greater numbers than L. cuprina.

Examination of the blowfly landings on the fish in the three trials demonstrates that pirimiphos-methyl exerted no repellent effect against blowflies. Similar blowfly counts were recorded on all treatments. In all trials, the blowfly counts on the fish decreased towards the end of the drying period.

Figures 4 to 6 show that fewer egg batches were oviposited on the pirimiphos-methyl treated fish than on the control fish. This observation differs from the results of previous trials which indicated that pirimiphos-methyl does not have a repellent effect against ovipositing blowflies. Treatment with pirimiphos-methyl did not prevent oviposition on the fish. However, examination of Figures 7 to 9 reveals that even if eggs were present on the pirimiphos-methyl treated fish, no subsequent larval infestation occurred. Close observation of the pirimiphos-methyl treated fish revealed that the eggs hatched out into tiny larvae which died within a short period. occasionally one or two large adult larvae were found to be present on the pirimiphos-methyl treated fish. These low levels of infestation were thought to be due to cross contamination from the infested, control fish.

Inspection of the fish after drying, revealed considerable damage to the untreated fish, which had suffered larval infestation. The pirimiphos-methyl treated fish only occasionally incurred larval infestation and this was generally at a low level. These fish, as a group, did not show any signs of insect damage and were generally of a superior quality compared with the control fish. This is confirmed by the damage assessment data shown in Figure 10.

Tables 1 to 3 show the weight losses at the various stages of processing. All trials contained treatments of equally matched fish weights before salting. In Trial A, the untreated fish lost about 2.3% more weight during drying than the Minawet treated fish (significant at the 1% level, Student t-test), presumably as a result of the damage suffered. In Trials B and C, no significant differences were found between weight losses of the various treatments.

Table 1

Trial A. Weight changes to fish during processing

	Control		Minawet	
	Mean	s.d.	Mean	s.d.
Wt. before salting (g)	1510	358	1521	351
% Loss during salting	15.5	4.0	15.3	3.0
% Loss during drying	39.1	2.1	36.8	3.3

22 fish per treatment

Table 2

Trial B. Weight changes to fish during processing

	Control		Minawet	
	Mean	s.d.	Mean	s.d.
Wt. before salting (g)	1506	534	1480	516
% Loss during salting	18.0	3.1	18.2	3.1
% Loss during drying	45.7	4.4	45.4	4.0

20 fish per treatment

Table 3

Trial C. Weight changes to fish during processing

	Control		Minawet	
	Mean	s.d.	Mean	s.d.
Wt. before salting (g)	1296	333	1281	266
% Loss during salting	33.6	6.7	31.5	7.7
% Loss during drying	24.4	7.8	27.5	5.7
	Minawet 10		Minawet 15	
	Mean	s.d.	Mean	s.d.
Wt. before salting (g)	1297	257	1285	253
% Loss during salting	35.1	8.1	32.2	6.6
% Loss during drying	25.4	8.1	26.6	8.5

10 fish per treatment

Pirimiphos-methyl is currently registered, as Minawet 250 EC, for use on cured fish in Indonesia. The recommended treatment is a 15 second immersion in a 0.03% a.i. emulsion of pirimiphos-methyl. In previous trials, this treatment has been shown to prevent infestation, while leaving residues on the fish of less than 10 mg/kg, the FAO/WHO maximum residue limit for pirimiphos-methyl. Trial C demonstrated that dipping times of 5 and 10 seconds were as effective as the 15 second dip in preventing blowfly infestation during processing. These results suggest that a shorter dipping time than has been recommended may be adequate to prevent infestation during processing. Further trials, using various, shorter dipping times need to be carried out to establish optimum conditions for Minawet application.

3. INSECT INFESTATION OF SALTED-DRIED FISH DURING STORAGE IN CILACAP, CENTRAL JAVA

3.1 Materials and Methods

Processing trials, to demonstrate the use of pirimiphos-methyl in protecting drying fish from blowfly infestation, had earlier been carried out on marine catfish (Arius sp.), in Cilacap, Central Java. After processing, some of the treated fish and untreated fish were stored for 14 weeks in order to assess whether treatment with pirimiphos-methyl before drying would give protection against insect infestation during storage, after drying. The storage trial was supervised by staff of the Fish Quality Control Laboratory in Cilacap, as part of the training program in post-harvest loss assessment and reduction.

The fish processing method consisted of the following steps: splitting, gutting, pickle curing for 2 nights and sun-drying for 2 days. Half of the fish were treated with pirimiphos-methyl by dipping in a 0.03% a.i. solution for 15 seconds before drying commenced. The other half of the fish, the controls, were dipped in water for 15 seconds before drying commenced.

After processing, the fish were stored in wooden boxes for 14 weeks. The treated catfish (27 fish) were placed in 3 boxes, the control catfish (27 fish) in 3 boxes. The boxes were placed in the storage area of the processor's salting shed in a row in order: C1 (control batch 1), T1 (treated batch 1), C2, T2, C3, T3.

After storage, the fish were individually weighed and inspected for insect infestation and damage. Damage was assessed according to the following scale:

Code	Observation
0	No visible damage
L	Occasional lesions to fish surface
M	Numerous scattered lesions
H	Large areas of damaged tissue

3.2 Results and Discussion

During storage, the untreated catfish suffered severe infestation by Piophila casei (see Table 4), with 26 out of 27 fish having larvae present. Adult and pupal stages of P. casei were also present on most of the untreated fish. Only 2 out of 27 of the pirimiphos-methyl treated fish had larvae present (very light infestation in both cases) and none had adults or pupae. The degree of infestation was reflected in the degree of damage observed, as seen in Table 4. Two-thirds of the untreated catfish showed medium or high damage, whereas no damage was observed for any of the pirimiphos-methyl treated fish.

All six batches of fish lost weight during storage. The untreated fish lost on average 5.1% more in weight during storage than the pirimiphos-methyl treated fish (significant at the 1% level, Student t-test), presumably as a result of the damage suffered.

Infestation with P. casei during storage of salted-dried fish had also been observed in earlier trials conducted in East Java on Arius, Lutjanus and Coryphaena species and it has been reported to be a widely distributed pest of salted, dried fish during storage (FAO, 1981).

4. INSECT INFESTATION OF SALTED, DRIED FISH DURING STORAGE IN MEDAN, NORTH SUMATRA

4.1 Materials and Methods

Processing trials, to demonstrate the effectiveness of pirimiphos-methyl in protecting drying fish from blowfly infestation, were carried out on red snapper (Lutjanus sebae) and grouper (Diagramma punctatum), at a fish processing site near Medan, North Sumatra. After processing, some of the treated fish and untreated fish were stored for 13 weeks to assess the protective effect of the pirimiphos-methyl treatment against insect infestation during storage. The storage trial was supervised by the staff of the Fish Quality Control Laboratory in Medan, as part of the training programme in post-harvest loss assessment and reduction.

The fish processing technique was the same for both species and consisted of splitting, gutting, brining overnight and sun-drying for 5 days. Half of the fish were treated with pirimiphos-methyl by dipping in a 0.03% a.i. solution for 15 seconds before drying commenced. The other half of the fish, the controls, were dipped in water for 15 seconds. During drying, the treated snapper had a mean weight loss of 48%, the control snapper a mean weight loss of 49%, the treated groupers a mean weight loss of 51% and the control grouper a mean weight loss of 53%.

After processing, the fish were stored in baskets for 13 weeks. The treated snapper (13 fish) were placed in 2 baskets, the control snapper (13 fish) in 2 baskets, the treated grouper (10 fish) in 2 baskets and the control groupers in 2 baskets. The baskets were placed in a random arrangement in the storeroom of a dried fish wholesaler in Medan.

After storage, the fish were individually weighed and inspected for insect infestation and damage. Damage was assessed according to the following scale:

Code	Observation
0	No visible damage
L	Occasional lesions on surface
M	Numerous scattered lesions
H	Large areas of damaged tissue

4.2 Results and Discussion

During storage, the untreated snapper suffered severe infestation by Dermestes maculatus Degeer (Table 5). All of the fish had shed, larval, dermestid skins present and almost half had Dermestes larvae and adults present. The treated snapper showed no insect infestation. The untreated grouper suffered less severe infestation than the snapper, with 80% having shed, larval skins present and 30% having Dermestes larvae present. The treated group showed no insect infestation.

The degree of infestation was reflected in the degree of damage observed (Table 5). Over one third of the untreated snapper showed medium or high damage; none of the treated fish showed any damage. Over three-quarters of the untreated grouper showed some damage, although only light. The treated grouper showed no damage, except for 2 fish, each of which had a single hole, caused presumably by Dermestes infestation.

Fish size appeared to be an important factor in determining the degree of infestation and damage. The grouper, which were in general smaller than the snapper, suffered lower degrees of infestation and damage. Amongst the snapper, the largest fish showed the highest degree of infestation and damage.

All four batches of fish lost weight during storage. The untreated snapper lost 8% more in weight during storage than the treated snapper (significant at the 1% level, Student t-test), presumably as a result of the damage suffered.

5. CONCLUSIONS

1. C. megacephala and L. cuprina were the two species of blowfly responsible for infestation of cured, marine catfish (Arius sp.) during processing trials conducted in Pontianak, W. Kalimantan.

2. Pirimiphos-methyl, applied as a 0.03% dip, effectively reduced infestation and prevented damage caused to Arius sp. by blowfly larvae during processing.

3. Dipping times of 5, 10 and 15 seconds were equally effective at controlling blowfly infestation and damage to Arius sp. during processing.

4. Untreated, salted, dried marine catfish (Arius sp.) suffered infestation with P. casei during storage resulting in severe damage during storage trials conducted in Cilacap, Central Java.

5. Treatment of the marine catfish with 0.03% a.i. pirimiphos-methyl after salting, but before drying, prevented insect damage during subsequent storage.

6. Untreated marine catfish lost 5.1% more weight on storage than pirimiphos-methyl treated fish, presumably due to P. casei damage.

7. Salted, dried snapper (L. sebae) suffered infestation with D. maculatus during storage, resulting in severe damage, particularly to large fish, during storage trials conducted in Medan, North Sumatra. Salted, dried grouper (D. punctatum), which were, in general, smaller in size than the snapper, suffered less severe infestation with D. maculatus and less severe damage.

8. Treatment of L. sebae and D. punctatum with 0.03% a.i. pirimiphos-methyl before drying proved effective in preventing Dermestes infestation and damage to L. sebae and greatly reducing infestation and damage to D. punctatum during storage.

6. ACKNOWLEDGEMENTS

The authors are grateful for the advice and assistance given by the following: Drs. Martono, Director of Enterprise and Processing Development, Directorate-General of Fisheries; Ir. Sukotjo Adisukresno, Head of the Fisheries Dept, Central Java; Ir. Djoko Sugiarto, Head of the Fisheries Dept, West Kalimantan; Ir. Boedi Soesilo, Head of the Fisheries Dept, North Sumatra.

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Fig. 1: Blowfly count per 22 fish and climatic data (Trial A)

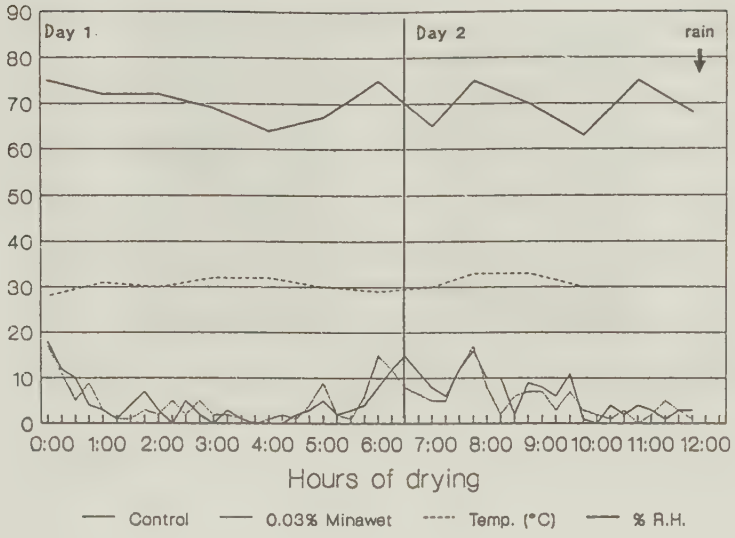


Fig. 2: Blowfly count per 20 fish and climatic data (Trial B)

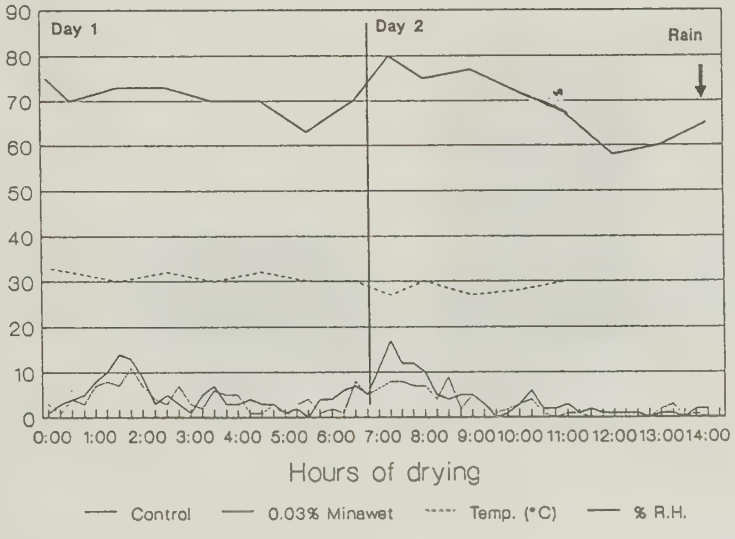


Fig. 3: Blowfly count per 10 fish in relation to Minawet dip times and climatic data (Trial C)

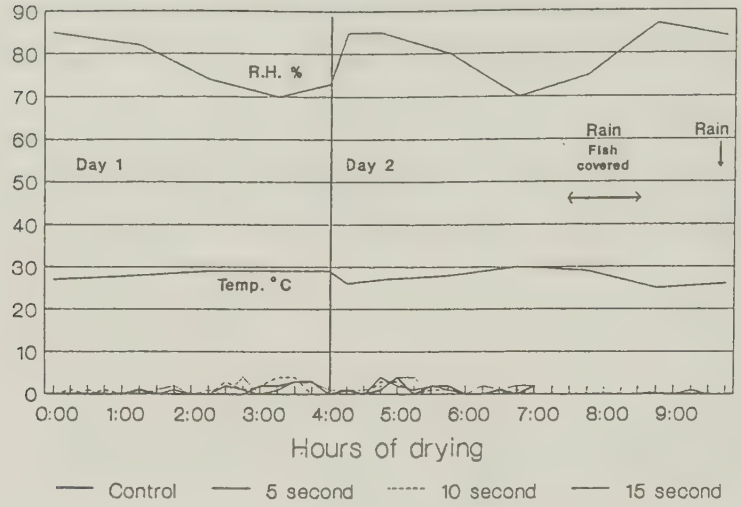
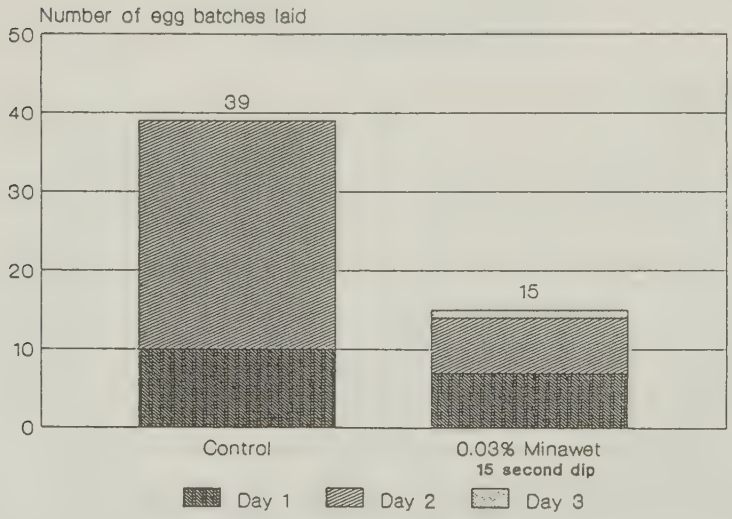
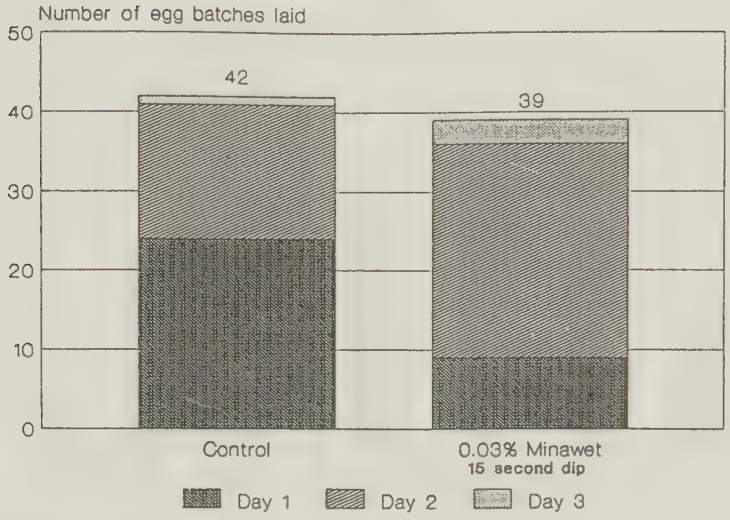


Fig. 4: Effect of Minawet on oviposition during drying. Trial A



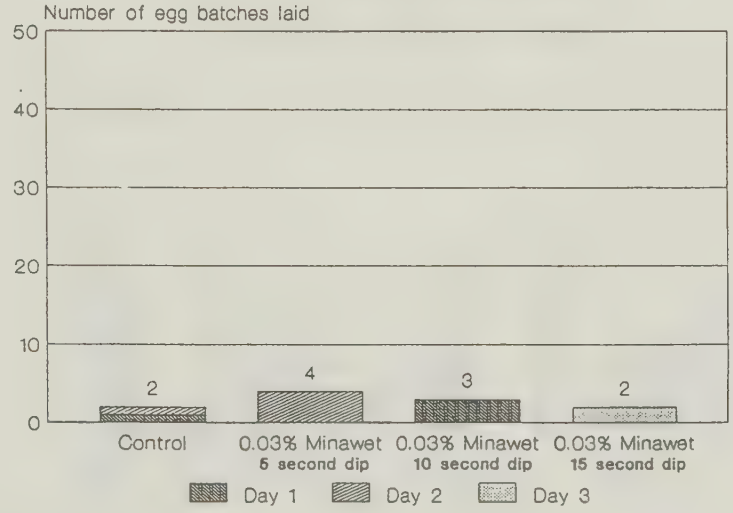
22 fish in each treatment

Fig. 5: Effect of Minawet on oviposition during drying, Trial B



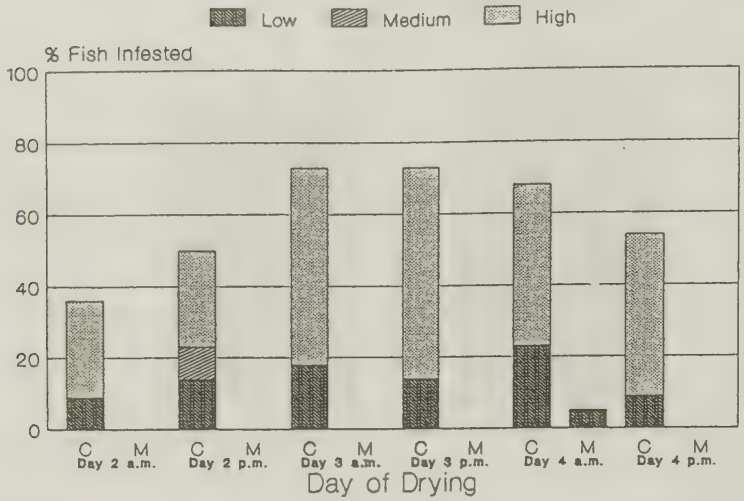
20 fish in each treatment

Fig. 6: Effect of Minawet on oviposition during drying, Trial C



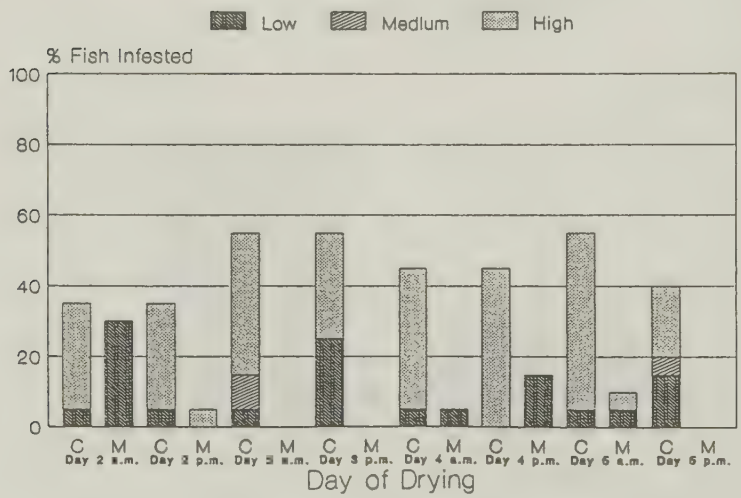
10 fish in each treatment

Fig. 7: Larval infestation during drying
Trial A



C=Control
M=0.03% Minawet 15 second dip

Fig. 8: Larval infestation during drying
Trial B



C=Control
M=0.03% Minawet 15 second dip

Fig. 9: Larval infestation during drying
Trial C

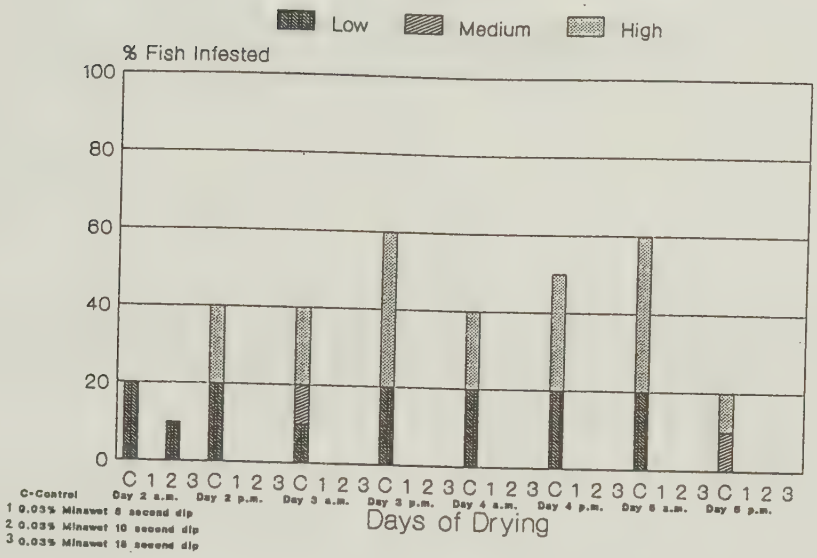
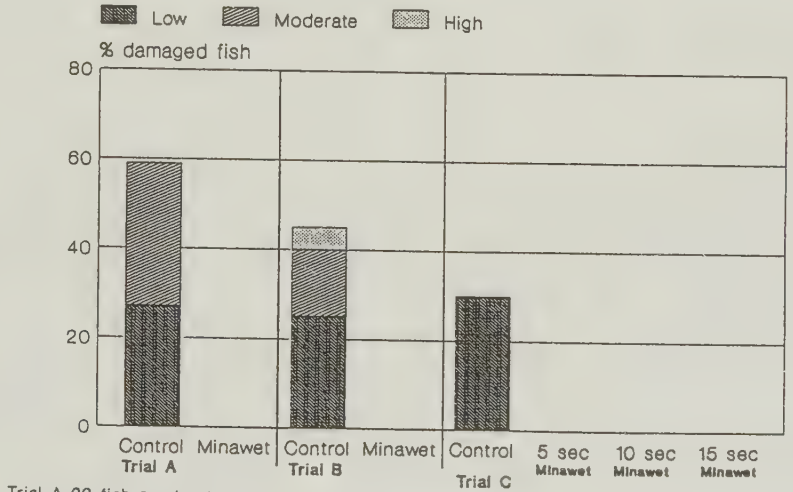


Fig. 10: Effect of Minawet on damage by blowfly larvae, Trials A, B and C



Trial A 22 fish per treatment
Trial B 20 fish per treatment
Trial C 10 fish per treatment

THE EFFECTS OF SALT CONCENTRATION ON OVIPOSITION AND LARVAL GROWTH AND MORTALITY IN THE BLOWFLY CHRYSOMYA MEGACEPHALA

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ABSTRACT

Field observations in Indonesia and Thailand have revealed that traditionally processed fish frequently become infested with blowfly larvae, which cause significant damage to the fish during both the salting and drying stages of processing. Chrysomya megacephala (Fabricius) is a common pest of salted, dried fish in S.E. Asia that can tolerate the relatively high salt concentrations which result from traditional salting and drying techniques.

Laboratory investigations, using cultures of C. megacephala established from pupae imported from Indonesia, have demonstrated, that when offered a choice of fish of different salt concentrations, mature, female flies will preferentially oviposit on the fish which has the lowest salt concentration. In the absence of choice, however, C. megacephala will readily oviposit on fish with high salt concentrations.

Trials on C. megacephala larvae have demonstrated that higher salt concentrations than those normally found in traditionally processed, salted, dried fish, are necessary to significantly reduce larval growth rate and increase larval mortality.

1. INTRODUCTION

Chrysomya megacephala (Fabricius) is a species of blowfly that is widely distributed throughout the Oriental and Australasian regions and also occurs in neighbouring parts of the Palaeartic region. It is commonly found near human dwellings and creates a great nuisance in fish market sand other areas where fish is handled (Zumpt, 1956). C. megacephala has also been reported in Ghana, Senegal and South Africa (Prins, 1979) and is one of a number of species of Diptera responsible for infesting fish during curing. It is a particularly serious pest of salted, dried fish in South East Asia and is a major cause of cured fish losses during processing. Field experiments, carried out in West Java, have indicated that C. megacephala will readily oviposit on fish, both during and after salting, and that C. megacephala larvae are tolerant of the high salt concentrations found in both the pickles used for curing, and in the salted fish itself (Esser, Hanson and Taylor, 1984). Subsequent observations in six provinces of Indonesia and three provinces of Thailand, suggest that salt tolerant C. megacephala is widely distributed in areas of South East Asia where fish are traditionally processed by salting and drying. In view of the consensus in the literature, that salting has an inhibitory effect on insect infestation and that heavy salting provides complete protection against blowfly larvae (FAO, 1981), it was decided to further investigate the relationship between fish salt concentration and blowfly infestation of salted fish, by carrying out a series of laboratory experiments using C. megacephala cultures established from pupae imported from W. Java, Indonesia. The experiments concentrated on the effects of fish salt content on C. megacephala oviposition and larval growth and mortality.

2. MATERIALS AND METHODS

2.1 Salting Treatment of Fish used as Oviposition and Feeding Medium

Cod (Gadus morhua) was used as the oviposition/feeding medium because it has a similar flesh appearance and texture to the flesh of marine catfish (Arius thalassinus), which is a commonly salted and dried species in South East Asia, and is particularly vulnerable to blowfly infestation during processing. The cod fillets were initially submerged overnight in freshwater and then submerged for a further night in brine solutions which ranged in salt concentration from 0% to 30% (w/v). This procedure was similar to the initial processing treatment carried out by traditional fish processors in West Java. After salting, the fish used for the oviposition experiments was cut into squares of approximately 16 cm^2, sealed in polythene bags and stored at -20°C. The fish used

in the larval growth and mortality experiments was minced and thoroughly mixed before being sealed in polythene bags and stored at -20°C. Before freezing, samples were taken randomly and analysed for salt and moisture content by using the methods described by FAO (1981). The salt and moisture contents of the 4 batches of fish used for the experiments are given in Table 1.

2.2 Maintenance of C. megacephala Cultures

The cultures were established from C. megacephala pupae imported from Indonesia and were maintained at temperatures of 28-30°C in cages constructed of an aluminium frame supporting a fine nylon mesh. The dimensions of the cages were 74 cm x 51 cm x 35 cm. Temperature was controlled by an overhead heat lamp, activated by a thermostat situated inside the cage. The heat lamp provided the only source of illumination during the trials.

The adult blowflies were reared from larvae that were fed on unsalted, fresh cod. After pupation, the newly emerged adults were fed daily on fresh, bloody, lamb's liver. Sucrose and water were constantly available to the flies.

Table 1

Relationship between the salt concentration of the brine used to salt the fish and the mean salt concentration (% dwb) and moisture content (%) of the fish after being salted

Fish batch	Brine concentration (%) 0	5	10	20	30
1. Moisture	83.8(0)	84.9(0.1)	82.7(0.9)	77.4(0.2)	73.4(0.5)
Salt	0.7(0)	13.4(0.4)	28.9(1.7)	39.5(2.1)	47.2(1.2)
2. Moisture	86.2(4.8)	85.6(0.3)	82.2(0.3)	77.6(0.9)	71.6(0.4)
Salt	1.1(0.4)	22.6(0.2)	33.8(0.7)	50.0(0)	53.5(0)
3. Moisture	81.7(0.4)	84.3(0.3)	82.0(0.3)	79.2(0.3)	74.6(0.5)
Salt	1.3(0.3)	14.4(0.4)	21.0(0.5)	33.2(0.6)	40.0(1.4)
4. Moisture	-	83.1(0.2)	81.7(0.5)	78.4(0.6)	77.4(0.8)
Salt	-	10.1(1.2)	14.6(5.3)	25.7(4.6)	30.0(0.6)

Standard deviations in brackets

2.3 Effect of Salt Concentration of Oviposition Medium on Ovi- position by C. megacephala

The investigation comprised five series of trials, designed to test the hypothesis that, irrespective of salt concentration, C. megacephala, when presented with fish of a range of salt concentrations, would preferentially oviposit on the fish with the lowest salt concentration, but that in the absence of choice, oviposition would still occur on heavily salted fish.

Each culture was maintained for 3-4 weeks before the trials commenced, to allow adequate time for the ovaries of the female adult flies to mature. The cultures were deprived of liver for 1 week before each series of trials commenced, to prevent premature oviposition and reduce the incidence of "spent" female flies.

Each series consisted of 4 trials, carried out on consecutive days. For the first trial of each series, the flies were presented with fish of 4 salt concentrations. In each succeeding trial, the choice of salt concentrations was progressively reduced by omitting the fish which had the lowest salt concentration in the previous trial. Hence, for the last trial of each series, the flies were only presented with fish of the highest salt concentration. The fish presented to the flies had previously been cut into 16 cm^2 blocks and each fish sample was placed in an open, plastic dish. Fish of each salt concentration was presented in triplicate and the dishes were placed in a fully random order on the floor of the cage. After 1 h the fish was removed from the cage and the number of eggs present on each fish sample was counted with the aid of a stereomicroscope.

The approximate numbers of flies present in the cultures used for the trials were as follows: Series A and B: - 2,000, Series C, D and E: - 1,500.

2.4 Effect of Salt Concentration of Feeding Medium on Growth and Mortality of C. megacephala larvae

A series of 5 trials was carried out over a period of 6 weeks, using laboratory bred C. megacephala larvae. The larvae used in the trials were reared on minced cod, of a range of salt concentrations, which had been processed and analysed using the methods described above.

For each trial, the larvae were reared on fish of five salt concentrations. The salt concentrations of the fish used for Trials 1 and 2 were 0.7, 13.4, 28.9, 39.5 and 47.2% (dwb). Trials 3, 4 and 5 used fish at salt concentrations of 1.1, 22.6, 33.8, 50.0 and 53.5% (dwb). At the start of each trial, 1g samples of fish of each salt concentration were placed on small dishes in separate, transparent, plastic jars. Each salt concentration was replicated four times. Twenty-five first instar larvae were then weighed and carefully transferred top each sample of fish. The jars were closed, using lids which contained 4 ventilation holes of 1 cm diameter. The holes were covered with fine, nylon mesh to prevent larvae from escaping. The fish was replaced daily, and from day 2 onwards, the quantity of fish used for each replicate was increased to 12g. The larvae were incubated at 30°C throughout the trials. At daily intervals, counts were taken of all surviving larvae and the larvae from two replicates of each salt concentration were weighed on a top-pan balance, accurate to 0.0001 g, until the onset of pupation.

3. RESULTS AND DISCUSSION

3.1 Effect of Salt Concentration of Oviposition Medium on Oviposition by C. megacephala

Table 2 shows that, with only one exception (Trial E.1), C. megacephala preferentially oviposited on the fish present with the lowest salt concentration. Oviposition was not, however, completely restricted to the fish present with the lowest salt concentration, suggesting that other factors may also influence choice of oviposition site. When choice of fish of different salt concentrations was restricted or absent, the flies readily oviposited on fish with relatively high salt concentrations of 30 to 40% (dwb). These salt levels exceed those usually found in traditionally processed fish in South East Asia. Salt concentrations, particularly in salted, dried fish prepared from large species, which are most susceptible to blowfly infestation, are usually less than 30% (dwb). It can also be seen from the standard deviations of the mean numbers of eggs present on the fish, that oviposition was not spread evenly between the 3 replicates of any given salt concentration. In general, once one fly had started ovipositing on a particular piece of fish, then other female flies would quickly join it and commence oviposition. This group oviposition would result in a large mass of eggs being deposited on a single piece of fish, whereas the other replicates of the same salt concentration would remain free of eggs. Group oviposition occurred during all of the trials and has also been frequently observed on fish being salted during field trials conducted in Indonesia.

The result that C. megacephala will readily oviposit on fish with relatively high salt concentrations differs from previously published opinion. Green (1967), Proctor (1977), Kordyl (1977) and McLellen (1963), all reported that salted fish were unattractive to blowflies. More recently, Walker and Wood (1985), found that salt appeared to inhibit egg laying on salted Haplochromis spp. and Lethrinops spp., during field trials carried out in Malawi. However, during Walker's trials, the blowflies found at the processing site were presented with a choice of unsalted fish and fish of salt concentrations of up to approximately 9%. The laboratory trials described in this paper and observations by the author at several cured fish processing sites in South East Asia, indicate that, in the absence of such a choice between salted and unsalted fish (which is generally the case at commercial processing sites), salting the fish to the salt levels usually found in South East Asian cured fish does not impart a repellent effect against ovipositing blowflies such as C. megacephala and Lucilia cuprina (Wied.).

3.2 Effect of Salt Concentration of Feeding Medium on Growth and Mortality of C. megacephala Larvae

Figures 1 and 2 show the effect of salt content on larval growth rate over the first three days of larval growth. observations at fish processing sites in S.E. Asia have demonstrated that it is during this period that blowfly larvae cause most damage to the fish. Figure 1 indicates that during the first two trials, a salt content of 28.9% (dwb) results in a reduction in growth rate of 14% and that a salt content of 39.5% (dwb) results in a reduction in growth rate of 54%. Although Figure 1 shows a more rapid decrease in growth rate between salt contents of 28.9% and 39.5% than was obtained by lower salt levels, the difference was not statistically significant due to the small number of samples tested. The results of Trials 3-5 (Figure 2) support the findings of the first two trials and also show that a salt content of 33.8% (dwb) gave a significant reduction in growth rate of 44% (Student t-test, $p<0.01$) and that a salt content of 50% gave a significant reduction in growth rate of 93% (Student t-test, $p<0.001$).

The larval growth curves given in Figures 3 and 4, show that the larvae reared on the unsalted fish grew most rapidly between the first and second days of the larval duration and underwent an approximately five-fold size increase within twenty-four hours. Salting the fish had the effect of

decreasing the initial growth rate, but over the complete larval duration, salt contents of less than 33.8% (dwb) had little effect on the total amount of larval growth.

The larval period of the fish reared on the unsalted fish lasted four days. Figures 3 and 4 show that salt concentrations of 47.2% and 50% (dwb) caused a delay in the onset of pupation. The few larvae that survived these high salt concentrations, continued to grow at a slow rate for a further three days before pupation commenced.

Figures 5 and 6 demonstrate the effect of salt content on larval mortality. The results show that a salt content of 47.2% (dwb) gave a significant increase in the mortality of C. megacephala larvae (Student t-test, $p<0.05$) and indicate that salt contents in excess of 39.5% are necessary to obtain high larval mortalities.

The laboratory trials demonstrated that the larvae of C. megacephala can tolerate higher salt concentrations than are normally present in traditionally processed, salted, dried fish. The results of these trials suggest that traditional fish processors would need to salt their fish to obtain salt contents of about 40% (dwb) in order to be reasonably certain of controlling infestation by C. megacephala. Although it would be possible to modify traditional salting techniques to obtain such high salt concentrations, observations in Indonesia suggest that the resulting, high salt content product would probably be unacceptable to both processors and consumers of salted, dried fish.

The findings that blowfly larvae can tolerate high salt concentrations again appears to contradict previously published reports. Kordyl (1977) observed that brining Lake Tanganyika sardines was sufficient to prevent the development of blowfly larvae and the Tropical Development and Research Institute (1984) reported that a salt concentration in excess of 8% gave larval control of at least 90% during trials conducted in Malawi.

Most of the published observations of blowfly attack on traditionally processed fish have been made in Africa, where fish are seldom salted before drying. Of the two species of blowflies observed to infest salted, dried fish in South East Asia, only L. cuprina has been reported to infest cured fish in Africa (Walker and Donegan, 1984). Although C. megacephala has been introduced to the African continent, it has not, as yet, been reported to cause problems to African fish processors. The apparent discrepancy between observations of blowfly oviposition behaviour and larval salt tolerance in Africa and South East Asia could be due to both C. megacephala and L. cuprina have evolved a tolerance to the relatively high salt concentrations found in traditionally processed, South East Asian fish, which is usually salted before drying. In Africa, where fish is not usually salted before drying, tolerance to high salt concentrations may not yet have been selected for in the blowfly populations associated with the indigenous, traditional fish processing sites. Should salting before drying be adopted as a method of controlling blowfly infestation in Africa, then the recent introduction of C. megacephala to that continent must present cause for serious concern. Further laboratory trials on blowfly species known to infest fish in Africa e.g. Chrysomya albiceps (Wied.), Chrysomya chloropyga putoria (Wied.) and Chrysomy regalis (Desvoidy), should be carried out to determine if salt tolerance is present in local blowfly species, before African processors are advised to adopt the relatively expensive and, probably locally unacceptable, technique of salting their fish before drying.

4. ACKNOWLEDGEMENTS

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Table 2

The effect of salt concentration on oviposition by C. megacephala

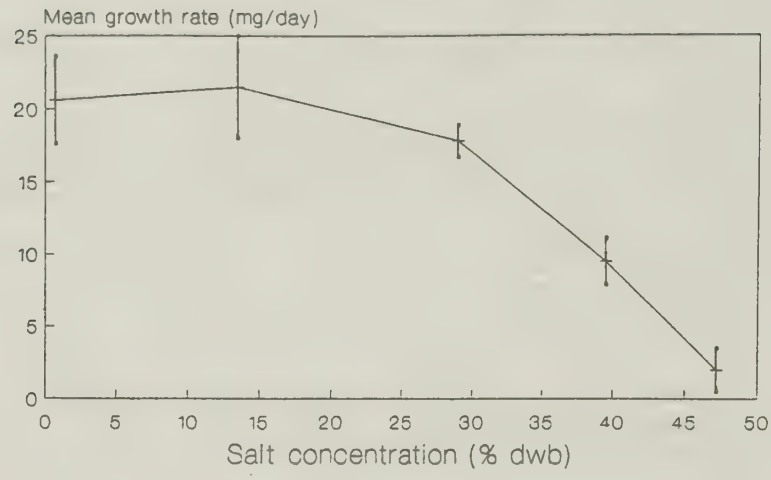
(a) Mean number of eggs oviposited on 3 replicates of each salt concentration during Series' A and B

	Salt concentration (% dwb)								% eggs on lowest salt
	14.4		21.0		33.2		40.0		
Trial	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	
A 1	1700	1864	62	84	0	0	0	0	97
2			98	96	0	0	0	0	100
3					234	210	0	0	100
4							771	1335	-
B 1	6512	2463	0	0	0	0	0	0	100
2			1236	2141	0	0	0	0	100
3					1025	1579	0	0	100
4							-	-	-

(b) Mean number of eggs oviposited on 3 replicates of each salt concentration during Series' C, D and E

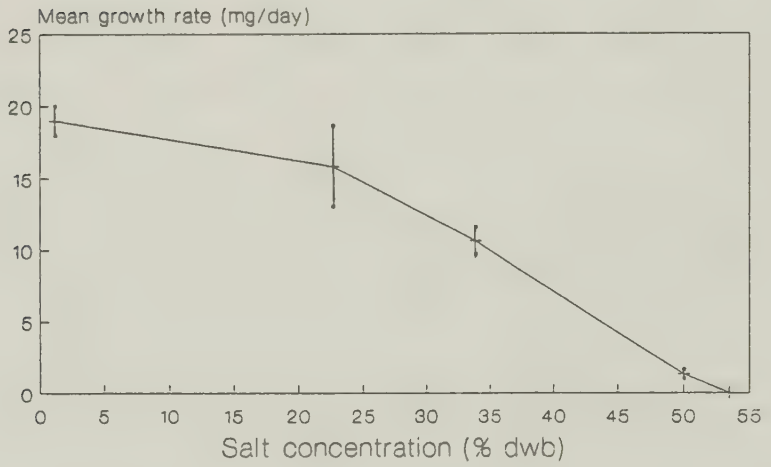
	Salt concentration (% dwb)								% eggs on lowest salt
	10.1		14.6		25.7		30.0		
Trial	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	
C1	734	1272	0	0	0	0	0	0	100
2			923	1180	0	0	0	0	100
3					3479	6026	0	0	100
4							1199	1267	-
C1	5737	5695	365	633	0	0	0	0	94
2			768	940	0	0	0	0	100
3					233	404	0	0	100
4							1219	2111	-
E1	580	606	52	90	679	1176	0	0	44
2			81	72	0	0	0	0	100
3					356	552	0	0	100
4							0	0	-

Fig.1: Effect of salt content on larval growth rate over first 3 days of growth.



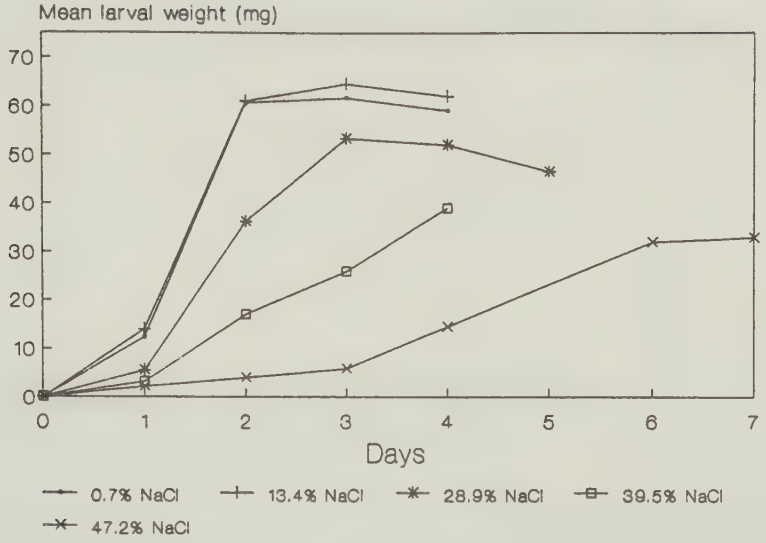
Trials 1 and 2 consolidated results.
Vertical intercepts=1 standard deviation

Fig.2: Effect of salt content on larval growth rate over first 3 days of growth



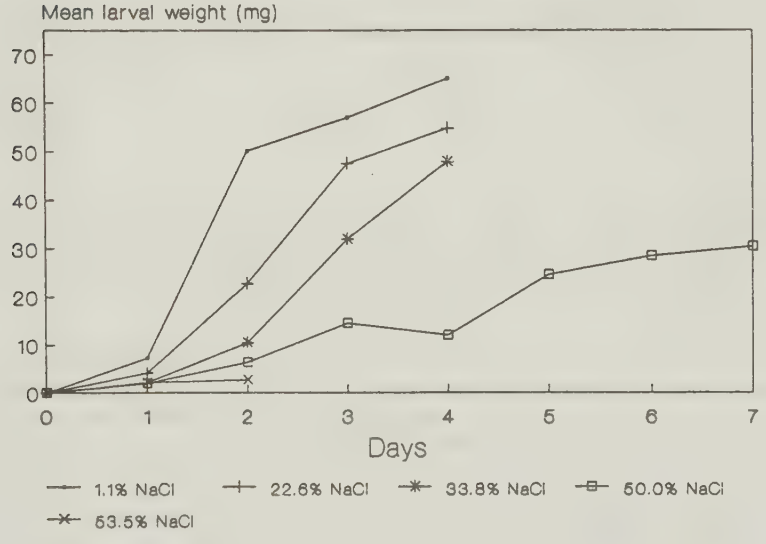
Trials 3-5 consolidated results
Vertical intercepts=1 standard deviation

Fig.3: Effect of salt content on amount of larval growth over larval duration



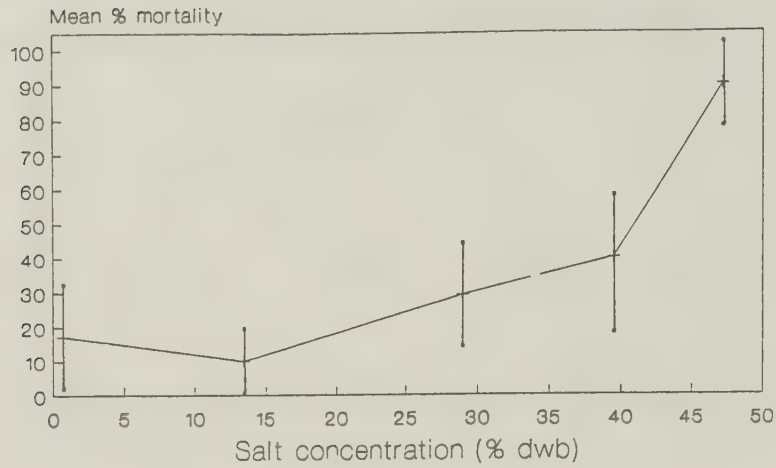
Trials 1 and 2 consolidated results.

Fig.4: Effect of salt content on amount of larval growth over larval duration



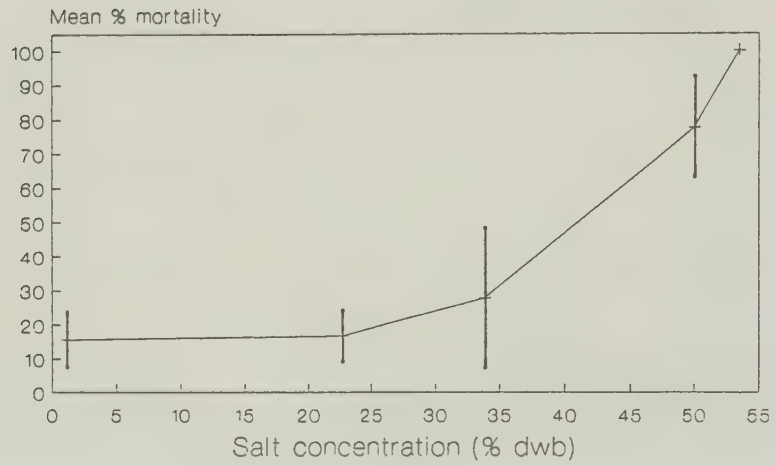
Trials 3-5 consolidated results

Fig.5: Effect of salt content on larval mortality



Trials 1 and 2 consolidated results.
Vertical intercepts=1 standard deviation

Fig.6: Effect of salt content on larval mortality



Trials 3-5 consolidated results.
Vertical intercepts=1 standard deviation

EVALUATION OF INSECTICIDES TO PROTECT SALTED DRIED MARINE FISH FROM INSECT INFESTATION DURING PROCESSING AND STORAGE IN THAILAND

by

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ABSTRACT

Trials to evaluate the use of insecticides in reducing insect infestation of traditionally processed fish were carried out in Samut Sakhon (west of Bangkok), Thailand, under rainy season conditions between October 1986 and February 1987. Selected samples were stored at tropical, ambient temperatures and evaluated after 15 weeks storage. Investigations were carried out on queenfish (Chorinemus lysan), marine catfish (Arius sp.), Spanish mackerel (Scomberomorus commersoni) and scad (Megalaspis cordyla), which are commonly processed in Thailand and known to suffer losses during processing and storage. Experimental work concentrated on monitoring insect infestation of cured fish samples treated with insecticides. Trials were carried out using pirimiphos-methyl, an organophosphorus insecticide, commercially known as "Actellic" or "Silosan" and deltamethrin, a pyrethroid insecticide commercially known as "Cislin. Insect damage, changes in weight, moisture and salt levels were also measured. Pirimiphos-methyl, applied as 0.03 and 0.06% a.i. dips and deltamethrin applied as a 0.003% dip protected the fish against blowfly infestation during processing. During storage all insecticide treatments provided protection against Dermestes infestation of salted, dried fish.

1. INTRODUCTION

Output of marine fisheries has increased dramatically in Thailand, following the rapid expansion of trawling fleets in fishing industry. Fish production has increased from 393,855 t in 1963 to 2.25 million t in 1983. The utilisation are as follows: fresh consumption 26.18%, frozen 6.9%, canning 5.16%, fishmeal 36.23%, animal feeding stuff and fertiliser 1.92%, curing 23.36% and others 0.25%.

About 23.36% of fisheries production is processing in the way which involves salting and sun-drying, smoking and fermentation. Most of these processing plants are still small-scale fishery communities and process the products by using traditional facilities. To look for the post-harvest problems and improve the quality of the traditional products, surveys were done in the central region where most of the processing plants are located and the North East region which are the big markets of these products. It was found that 7-12% of production was lost due to insect infestation. Moreover, the use of unproved insecticides by processors and retailers are also widespread. At the present time this infestation problem is being ameliorated in small fish processing operations in Thailand by use of the organophosphorus insecticide Dipterex (Dimethy 2,2,2-trichloro-1-hydroxyethylphosphate) which although approved for agricultural and horticultural use is not approved for use on fish by FAO/WHO. Use of alternative methods of insect control such as screening do not appear to be practised in Thailand at present.

Pirimiphos-methyl has been given approval for use on dried fish in Malawi and is currently being considered for use on fish in Indonesia. Pyrethroid insecticides such as deltamethrin and cypermethrin, while not at present permitted for use on fish, have been demonstrated to be very effective in controlling insect infestation at low concentrations (Esser et al. 1986).

Six processing trials were carried out at two fish processing sites in Samut Sakhon during October 1986 (rainy season). Samples from each trial were then stored under tropical, ambient conditions at two sites and evaluated during February 1987, after 15 weeks storage.

The main aims of the trials were:

a) To obtain further information on the causes of losses to traditionally processed fish in Thailand and to investigate the use of unapproved insecticides by fish processors.

b) To evaluate the use of pirimiphos-methyl and deltamethrin in the prevention of insect infestation and subsequent losses during the processing and storage of traditionally processed fish.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Fish

All fish were purchased fresh from local fish landing sites in Samut Sakhon, and delivered, packed in ice, to the fish processor on the morning of purchase. Fish purchased were:

Catfish (Arius sp.)
Scad (Megalaspis cordyla)
Spanish mackerel (Scomberomorus commersoni)
Queenfish (Chorinemus lysan)

2.1.2 Insecticides

The insecticide formulations used in the trials were as follows:

a) Pirimiphos-methyl (Actellic, Silosan). An organophosphorus insecticide 2-diethylamino-6-methylpirimidin-4-yl dimethyl phosphorothionate ($C_{11}H_{19}N_3O_3PS$), supplied by ICI Plant Protection Division plc, U.K. as Actellic 40% emulsifiable concentrate.

b) Deltamethrin. A pyrethroid insecticide - supplied by Wellcome Foundation Ltd, U.K. as Cislin 1% suspension concentrate.

2.2 Methods

2.2.1 Fish processing and storage

Specific details of individual trials are described later. However, the general scheme followed for trials SSA to SSE was as follows:

a) Fish gutted and beheaded on arrival.
b) Fish placed in a brine solution for 24 h.
c) Fish removed from brine and then soaked in water for about one hour.
d) Fish drained and insecticide treatments applied.
e) Samples sundried on racks for two to three days.
f) Dried fish transferred to paper bags or bamboo baskets and stored for 15 weeks under tropical, ambient conditions.

Trial SSF on smoked catfish involved the following process:

a) Fish gutted and washed on arrival.
b) Sun-dried for one day (without use of salt).
c) Hot-smoked for 12 h (overnight).
d) Insecticide treatments applied, and fish dried for approximately 3 h.
e) Fish then wrapped in paper and stored in bamboo baskets for 15 weeks under tropical, ambient conditions.

2.2.2 Salt and moisture analysis

The standard methods described in FAO (1981) were used on all samples.

2.2.3 Assessment of insect infestation during processing

The fish were examined for blowfly eggs and larvae after salting and at the end of each day's drying.

Larval infestation was assessed according to the following grading system.

Code	Observation
0	No larvae
L	Occasional larvae
M	Numerous larvae (no feeding packs)
H	Feeding packs

Blowfly activity over the drying fish was monitored by taking blowfly counts at 15-30 minute intervals throughout the drying period.

2.2.4 Assessment of insect damage during processing

At the end of drying, a visual assessment of damage was made based on the following grading system:

Code	Observation
0	No sign of larval damage
L	Traces of larval damage
M	Obvious signs of larval damage
H	Fish badly damaged by larvae

2.2.5 Assessment of insect infestation and damage during storage

The fish were individually weighed and examined for the presence of Dermestes larvae, adults and larval casts. Samples of adults were collected for identification. Damage was assessed according to the following grading system.

Code	Observation
0	No damage
L	Occasional holes in flesh
M	Numerous holes but flesh not completely eaten out
H	Flesh completely eaten out

Photographs illustrating the different damage levels were also taken.

2.2.6 Insecticide application

Two application procedures were used.

a) Trials SSA-SSE

After the fish had been brined, washed and drained, the following insecticide treatment was applied. The fish were individually weighed and sorted into four batches, ensuring that each batch contained a similar distribution of fish weights. The four batches were then treated as follows:

Control batch

Fish were submerged for 15 seconds in 6 L of fresh water and drained.

0.03% Pirimiphos-methyl solution treated batch

Fish were submerged for 15 seconds in 6 L of 0.03% pirimiphos-methyl and drained.

0.06% Pirimiphos-methyl solution treated batch

Fish were submerged for 15 seconds in 6 L of 0.06% pirimiphos-methyl and drained.

0.003% Deltamethrin solution treated batch

Fish were submerged in 6 L of the insecticide solution for 3 seconds, allowed to drain for one second and then resubmerged for a further 3 seconds before being placed on the drying racks.

To ensure that the fish did not deplete the active ingredient in the individual insecticide solutions, not more than 15 fish were dipped in any one preparation.

The above regime is taken from the scheme devised and developed for use in Indonesia (Esser et al., 1986).

b) Trial SSF

After the fish had been hot-smoked the 0.03% and 0.06% pirimiphos-methyl solutions and the 0.003% deltamethrin solution were applied as follows.

The fish were weighed and randomly divided into four equal batches of 50 fish each, three for insecticide treatment and one control batch. 500 mL of each insecticide solution was prepared and transferred to a hand operated spray gun. Batches of fish were treated with 80 applications of spray of a prepared solution of insecticide (40 applications to each side of each fish batch). The weight of insecticide used per batch was measured by weighing the spray gun before and after use (insecticide weight used varied within 21-27 g). After spraying, the smoked fish were sun-dried for a three hour period before being wrapped in paper and stored in bamboo baskets for the storage trials.

2.3 Individual Processing Trials

2.3.1 Trial SSA (Site 1) - Catfish (Arius sp.)

100 fresh, iced fish were examined on arrival for visual defects and fish temperatures were recorded. All fish were then gutted, beheaded and individually weighed and tagged before being transferred to a wooden tank and covered with a brine solution made from:

Sea salt (3 baskets)
Water (4 baskets)
Red dye/"Nitrate" mixture (50 g)

The fish were left for 18 h in the brine tank containing 25.4% NaCl and were then removed, weighed and soaked in fresh water for one hour.

They were then divided into four equal batches, ensuring that each batch contained approximately equal quantities of fish. The fish were then dipped in insecticide solutions as described under 2.2.6, and transferred to the drying trays which were placed out in the sun in a randomised sequence. The fish were dried for two days (0900-1600 h per day, covered overnight), reweighed after drying and examined for evidence of egg and larval infestation.

All dried fish from each batch were then placed in separate strong, paper bags and stored at tropical, ambient temperature for 3 months, after which they were re-examined for infestation. Randomly selected samples were analysed at the Fishery Technological Development Laboratory for moisture and salt using the standard methods described in 2.2.2.

2.3.2 Trial SSB (Site 1) - Scad (M. cordyla)

100 fresh, iced fish were examined for visual defects and individual weights were recorded. As the fish were of similar size they were not individually tagged. The whole fish were then placed in a brine solution (as in Trial SSA) and left in this solution for 2 days before being removed, weighed and washed in fresh water. The fish were then divided randomly into four equal batches and further experimental work carried out as described for Trial SSA>

2.3.3 Trial SSC (Site 1) - Spanish mackerel (S. commerson)

48 fresh, iced fish were examined for visual defects and fish temperatures were recorded. All fish were gutted by physically pulling out the gut and gills by hand, and were then individually weighed and tagged. The gutted fish were placed in a brine solution as in Trial SSA, and left in this solution for 2 days before being removed weighed and washed in fresh water. Further experimental work was carried out as described for Trial SSA.

2.3.4 Trial SSD (Site 2) - Queenfish (C. lysan)

12 fresh, iced fish were examined for visual defects, split and gutted, placed in a tank (1 m^3) and immersed in a saturated solution of salt for 24 h. The fish were removed, washed in fresh water and filleted. The fillets were divided into four equal batches, ensuring that each batch contained a similar distribution of weights. Further experimental work was carried out as described for Trial SSA.

2.3.5 Trial SSE (Site 2) - Catfish (Arius sp.)

48 fresh, iced fish were examined for visual defects and the whole fish were placed in a tank (1 m^3) and immersed in a saturated solution of salt for 24 h. The fish were removed, washed in fresh water and filleted. The fillets were divided into four equal batches, ensuring that each batch contained a similar distribution of weights. Further experimental work was carried out as described for Trial SSA.

2.3.6 Trial SSF (Site 2) - Smoked catfish (Arius sp.)

200 fresh catfish were hot smoked overnight (12 h) and further experimental work carried out as described in 2.2.6.

3. RESULTS AND DISCUSSION

Weight losses for fish batches used in Trials SSA to SSF are presented in Tables 1 to 6. Within each trial, mean percentage weight losses per treatment, due to dehydration, were not significantly different, i.e. as expected, different treatments had no measurable effect on relative weight losses. In trials SSA to SSC, percentage moisture by chemical analysis corresponded approximately with the percentage weight losses after drying. However, in trial SSB, the percentage moisture by analysis of 54.8% does not appear to correlate well with the low mean percent weight loss of only 8.2-9.5%. This could be due to a combination of relatively high lipid content and the fact that this species is not gutted during processing.

Chrysomya megacephala was the only blowfly species observed at the processors' premises during the course of the trials and, with the exception of one day (14th October), was present in very low numbers. The pattern of blowfly activity seen in Figure 1 is similar to that observed during earlier trials in Indonesia where the number of blowfly settling on the drying fish tended to increase during the late afternoon. It can also be seen that of the insecticide treatments, only the 0.003% deltamethrin had a repellent effect on blowfly. These results support earlier findings in Indonesia when pirimiphos-methyl and a similar pyrethroid insecticide, cypermethrin, were evaluated (Esser et al., 1986). Blowfly activity during these trials seemed to be influenced by weather changes. On most occasions the weather was hot (33^{o}-38^{o}C) and sunny, the fish dried rapidly and blowfly activity was consequently low. A build up of cloud cover from 3 pm onwards on 14th October was accompanied by a significant increase in blowfly activity on the fish drying in trial SSE. The processor reported that the blowfly problem is always worse on dull, still, humid days and that during extended periods of such weather his fish would be liable to blowfly infestation unless protected by insecticide application. Similar behaviour has been observed during trials in Indonesia although no statistically significant correlation between numbers of blowfly and weather have been demonstrated due to the difficulty in imposing adequate experimental controls under typical fish processing conditions. Another factor which could explain the generally low level of blowfly activity is the widespread, prophylactic use of insecticides, such as Dipterex, by the fish processing community which regards blowflies as a major threat to the fish. Such widespread use of insecticides on fish could, conceivably, over a period of time, result in a decrease in the local blowfly population in the absence of suitable, alternative breeding media for the flies.

Egg laying and infestation with blowfly larvae (Table 6) was restricted to the S. commersoni processed in trial SSC.

The eggs were either deposited in the abdominal cavity or under the opercula and resulted in 92% infestation of the control fish. All insecticide treatments gave complete protection against larval infestation during this trial.

During storage the fish were subjected to dermestes beetle attack, particularly at the Samut Sakhon site. Of the 27 beetle samples collected from the stored fish, 22 were identified as Dermestes maculatus and five as D. ater. Occasionally Necrobia rufipes were also observed on the fish.

Levels of infestation of the salted, dried fish left in store for six weeks at the Tatien market, Bangkok were lower than that observed at the processor's premises in Samut Sakhon. The Arius processed in trial SSA showed no evidence of beetle infestation and the mean weight loss of 46.5% (Table 7) during storage was solely due to dehydration. Moisture content of the SSA fish dropped from a mean of 55.9% to 17.5% during storage and mean salt content was 29% (dwb).

There was some evidence of Dermestes infestation of the M. cordyla (Trial SSB) during storage although damage caused was very light (Table 8). The fish showed a mean weight loss of 40.25% due to dehydration during storage. Mean moisture content decreased from 54.8% to 34.7% and mean salt content was 20% (dwb). The 0.03% pirimiphos-methyl treatment was effective in reducing infestation and complete protection against infestation was given by the 0.06% - pirimiphos-methyl and 0.003% deltamethrin treatments.

The S. commersoni (Trial SSC) remained completely free from insect infestation during storage and a mean weight loss of 52.5% due to dehydration was observed during storage (Table 9). Mean moisture content decreased from 61.1% to 14.5% and the mean salt content was 27% (dwb).

The C. lysan (Trial SSD) left in store at Samut Sakhon showed evidence of heavy infestation of the control fish during early storage although damage caused was slight (Table 10). A mean weight loss of 39.5% due to dehydration was observed and mean moisture content decreased to 15.7%. The

0.03% pirimiphos-methyl treatment again reduced infestation and the 0.06% pirimiphos-methyl and 0.003% deltamethrin treatments once more imparted complete protection against infestation.

Of the salted dried fish left in store, the Arius sp. (Trial SSE) showed most evidence of dermestes damage (Plate 1). The control fish suffered a decrease in yield of approximately 5% compared with the insecticide treated fish and 75% showed moderate to high beetle damage (Table 11). The 0.03% and 0.06% pirimiphos-methyl treatments reduced beetle infestation and damage and the 0.03% deltamethrin again provided complete protection. The mean moisture content after 15 weeks storage was 27.7% and salt content was 14% (dwb).

The smoked Arius (Trial SSF) suffered the heaviest beetle infestation and damage during storage (Table 12). All of the control fish suffered moderate to high insect damage (Plate 2) which resulted in a decrease in yield of approximately 25%. The low moisture content of 9.79% was undoubtedly a factor in making this product so vulnerable to beetle infestation. The results of the 0.03% and 0.06% pirimiphos-methyl treatments are ambiguous in that the 0.03% treatment appeared to give better protection than the 0.06% treatment which gave results very similar to the control group. Of the two insecticides tested the 0.003% deltamethrin treatment gave the best protection and appeared to have a repellent effect against beetles. The unusual results of the pirimiphos-methyl treatments could be explained by the fact that during storage the baskets of fish were moved by the processor to facilitate building work. It is possible that the basket containing the 0.03% pirimiphos-methyl treated fish was placed in close proximity with the basket containing the deltamethrin treated fish and that the deltamethrin exerted a repellent effect against beetles which otherwise might have infested the fish treated with 0.03% pirimiphos-methyl. Beetle infestation is a major problem of smoked fish in Thailand and in view of the promising results obtained with the deltamethrin treatment it is recommended that this pilot experiment be refined and repeated on a larger scale under more controlled and regularly monitored storage conditions.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Conclusions

1) Blowfly activity was low during the processing trials and resulted in only the S. commersoni becoming infested with blowfly larvae.

2) Pirimiphos-methyl applied as a 15 second, 0.03% dip and deltamethrin applied as a 6 second, 0.003% dip protected S. commersoni against blowfly infestation during processing.

3) Blowfly infestation is regarded as a major problem by fish processors in Thailand. The use of unapproved insecticides, in particular trichlorphon (Dipterex) is widespread in the fish processing communities of Samut Sakhon and Songkhla and is reported to be common practice in other parts of Thailand.

4) Infestation of D. maculatus and D. ater resulted in heavy damage and physical losses of approximately 25% in smoked Arius sp. (marine catfish) left in store over a 15 week period.

5) A pilot experiment indicated that a spray application of 0.003% deltamethrin is effective in preventing dermestid beetle infestation of smoked catfish over a 15 week storage period.

6) A 0.03% pirimiphos-methyl dip reduced dermestid beetle infestation of salted, dried fish over a 15 week storage period.

7) Both 0.06% pirimiphos-methyl and 0.003% deltamethrin dips gave complete protection against dermestid beetle infestation of salted, dried fish over a 15 week storage period.

4.2 Recommendations

1) Further information on the extent of the insect infestation problem should be collected from traditional fish processors, wholesalers and retailers in different regions of Thailand.

2) Loss assessment and reduction trials, involving both insecticidal and non-insecticidal (e.g. screening) methods, should be carried out on a variety of traditionally processed fish products in different regions of Thailand.

3) Traditional fish processors should be made aware of the potential hazards of using unapproved insecticide on their products and receive training in safe, alternative loss reduction methods.

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Mrs P. Xumsai	British Embassy, Bangkok

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Table 1

Weight loss of catfish (Arius sp.) after salting and after drying (Trial SSA)

Treatment	Mean % Wt Loss after salting	s.d.	Mean % Wt Loss after drying	s.d.
Control	10.8	2.7	41.2	4.7
0.03% pirimiphos-methyl	11.7	2.6	44.2	3.7
0.06% pirimiphos-methyl	13.5	2.6	43.0	4.4
0.003% deltamethrin	11.9	2.8	42.5	4.3

Note: Chemical composition of dried fish

Moisture = 55.9%
Salt = 12.9% wet wt. basis
= 29.3% d.w.b.

Chemical composition of brine used

% salt (NaCl) = 25.4%

Table 2

Weight losses of scad (M. cordyla) after drying (Trial SSB)

Treatment	Mean fresh weight	s.d.	Mean weight after drying	s.d.	Mean weight loss
Control	155.0	20.1	142.0	19.0	8.4
0.03% pirimphos-methyl	162.1	20.9	148.8	19.7	8.2
0.06% pirimiphos-methyl	156.2	24.3	142.4	22.9	8.8
0.003% deltamethrin	154.2	19.7	139.6	18.4	9.5

Note: Chemical composition of dried fish

Moisture = 54.8%
Salt = 9.2% w.w.b.
= 20.4% d.w.b.

Table 3

Weight losses of spanish mackerel (S. commersoni) after salting and after drying (Trial SSC)

Treatment	Mean % wt. loss after salting	s.d.	Mean % wt. loss after drying	s.d.
Control	12.9	1.4	28.0	2.4
0.03% pirimiphos-methyl	12.1	1.2	27.4	2.1
0.06% pirimiphos-methyl	12.3	1.3	27.8	1.6
0.003% deltamethrin	12.4	1.4	27.7	3.4

Note: Chemical composition of dried fish

Moisture = 61.1%
Salt = 10.4% w.w.b.
= 26.7% d.w.b.

Chemical composition of brine used

% salt (NaCl) = 30.6%

Table 4

Weight losses of queenfish (C. lysan) after salting and after drying (Trial SSD)

Treatment	Mean % wt. loss after salting	s.d.	Mean % wt. loss after drying	s.d.
Control	4.0	1.7	32.9	2.8
0.03% pirimiphos-methyl	4.7	1.0	34.9	1.9
0.06% pirimiphos-methyl	5.5	3.0	34.6	2.2
0.03% deltamethrin	5.2	1.6	35.1	1.5

Table 5

Weight losses of catfish (Arius sp.) after drying, compared to weights after salting (Trial SSE)

Treatment	Mean % wt. loss of fillets after drying	s.d.
Control	47.7	3.4
0.03% pirimiphos-methyl	47.9	2.6
0.06% pirimiphos-methyl	47.3	3.7
0.003% deltamethrin	48.1	3.9

Table 6

Blowfly infestation of S. commersoni during processing (Trial SSC)

Treatment	Number of egg batches	% larval infestation 0	L	M	H
Control	3	8	42	17	33
0.03% pirimiphos-methyl	1	100	0	0	0
0.06% pirimiphos-methyl	0	100	0	0	0
0.003% deltamethrin	0	100	0	0	0

Table 7. Salted, dried Arius (Trial SSA) data collected at the end of storage

Treatment	Weight before storage (g)			Weight after storage (g)			% Weight change	No. Dermestes larvae	No. Dermestes adults		% Fish with numerous casts	% Damaged Fish		
	$\bar{x}$	σ_{n-1}	Σx	$\bar{x}$	σ_{n-1}	Σx			Live	Dead		0	L	M
Control	393	159	8647	201	74	4426	-49	0	0	0	0	100	0	0
0.03% Pirimiphos Methyl	367	159	9185	211	85	5268	-42	0	0	0	0	100	0	0
0.06% Pirimiphos Methyl	80	179	9500	193	80	4827	-49	0	1	0	0	100	0	0
0.003% Deltamethrin	377	156	9414	202	81	5054	-46	0	0	0	0	100	0	0

Storage location - Tatien Market, Bangkok

Table 8. Salted, dried *M. cordyla* (Trial SSB) data collected at the end of storage

Treatment	Weight before storage (g)			Weight after storage (g)			% Weight change	No. Dermestes larvae	No. Dermestes adults		% Fish with numerous casts	% Damaged fish			
	$\bar{x}$	σ_{n-1}	Σx	$\bar{x}$	σ_{n-1}	Σx			Live	Dead		0	L	M	H
Control	142	19	3549	85	14	2136	-40	0	0	0	0	68	23	9	0
0.03% Pirimiphos Methyl	149	20	3721	87	12	2164	-42	0	0	0	0	96	4	0	0
0.06% Pirimiphos Methyl	142	23	3561	86	16	2160	-39	0	0	0	0	100	0	0	0
0.03% Deltamethrin	140	18	3491	84	12	2112	-40	0	0	0	0	100	0	0	0

Storage location - Tatien Market, Bangkok

Piophila pupal cases and occasional Dermestes larval cases present on control fish

Table 9. Salted, dried *S. commerson* (Trial SSC) data collected at the end of storage

Treatment	Weight before storage (g)			Weight after storage (g)			% Weight change	No. Dermestes larvae	No. Dermestes adults		% Fish with numerous casts	% Damaged fish			
	$\bar{x}$	σ_{n-1}	Σx	$\bar{x}$	σ_{n-1}	Σx			Live	Dead		0	L	M	H
Control	391	117	4698	190	63	2277	-52	0	0	0	0	100	0	0	0
0.03% Pirimiphos Methyl	388	97	4662	184	47	2068	-56	0	0	0	0	100	0	0	0
0.06% Pirimphos Methyl	379	70	4547	197	31	2360	-48	0	0	0	0	100	0	0	0
0.03% Deltamethrin	371	57	4452	173	25	2073	-53	0	0	0	0	100	0	0	0

Storage location - Tatien Market, Bangkok

Table 10. Salted, dried *C. lysan* (Trial SSD) data collected at end of storage

Treatment	Weight before storage (g)			Weight after storage (g)			% Weight change	No. Dermestes larvae	No. Dermestes adults		% Fish with numerous casts	% Damaged fish			
	$\bar{x}$	σ_{n-1}	Σx	$\bar{x}$	σ_{n-1}	Σx			Live	Dead		0	L	M	H
Control	2063	665	6185	1170	344	3510	-43	0	0	0	100	0	100	0	0
0.03% Pirimiphos Methyl	1787	245	5362	1064	154	3192	-40	0	10	0	0	67	33	0	0
0.06% Pirimiphos Methyl	1847	266	5540	1991	119	3573	-36	0	0	1	0	100	0	0	0
0.003% Deltamethrin	1584	671	4752	955	386	2864	-40	0	0	0	0	100	0	0	0

Storage location - premises of Mr Mink, Samut Sakhon

Table 11. Salted, dried Arius (Trial SSE) data collected at the end of storage

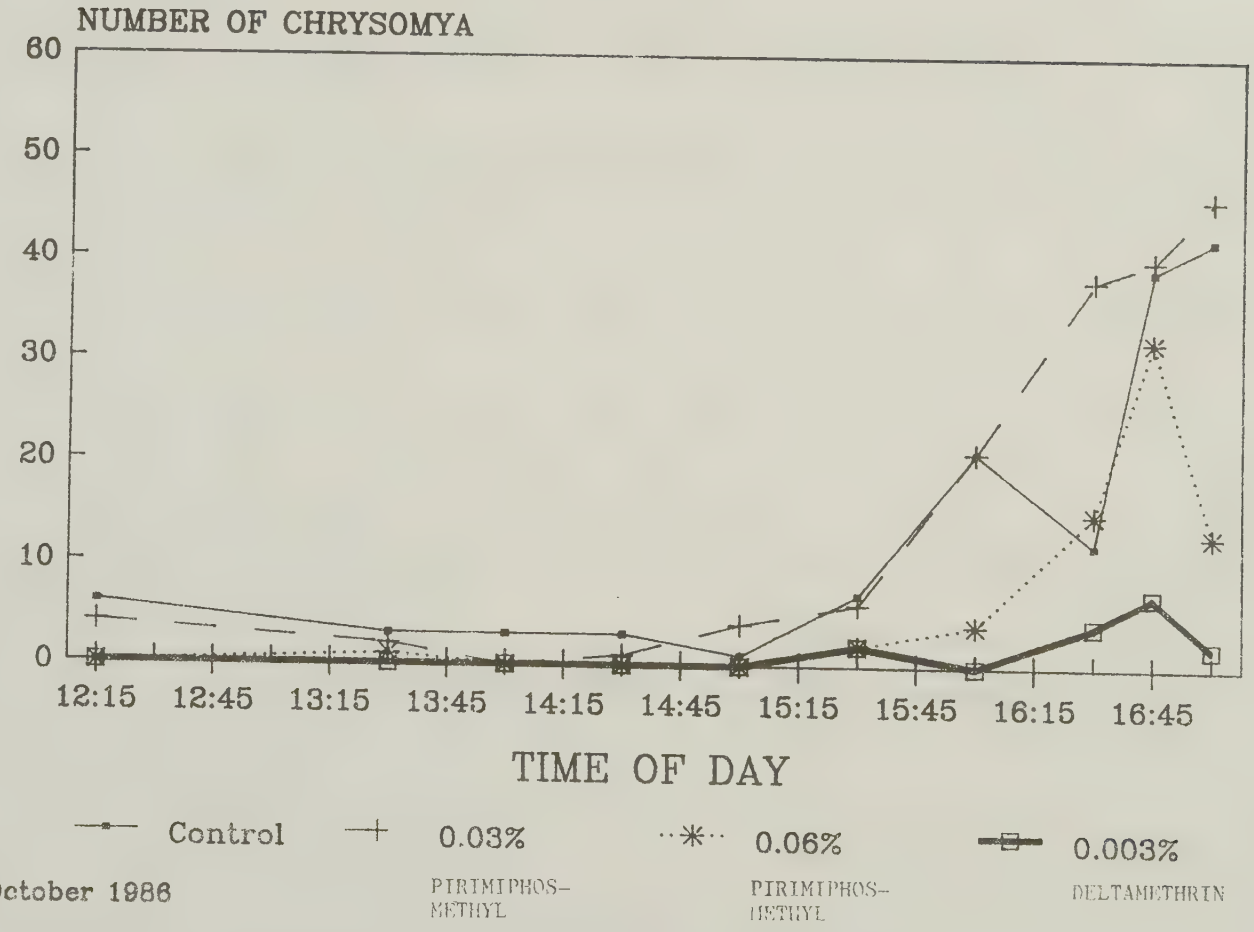
Treatment	Weight before storage (g)			Weight after storage (g)			% Weight change	No. Dermestes larvae	No. Dermestes adults		% Fish with numerous casts	% Damaged fish			
	$\bar{x}$	σ_{n-1}	Σx	$\bar{x}$	σ_{n-1}	Σx			Live	Dead		0	L	M	H
Control	186	56	2235	105	23	1261	-44	8	0	0	33	17	8	33	42
0.03% Pirimiphos Methyl	185	48	2225	117	25	1400	-37	0	1	2	8	50	8	42	0
0.06% Pirimiphos Methyl	186	43	2238	116	23	1394	-38	0	1	10	0	75	25	0	0
0.003% Deltamethrin	185	51	2224	112	23	1348	-39	0	0	0	0	100	0	0	0

Storage location - premises of Mr Mink, Samut Sakhon

Table 12. Smoked Arius (Trial SSF) data collected at end of storage

	Weight before storage (g)			Weight after storage (g)			% Weight change	No. Dermestes larvae	No. Dermestes adults		% Fish with numerous casts	% Damaged fish			
									Live	Dead					
	$\bar{x}$	σ_{n-1}	Σx	$\bar{x}$	σ_{n-1}	Σx						0	L	M	H
Control	74.6	34.3	3728	57.3	25.8	2807	-25	49	92	2	86	0	0	6	94
0.03% Pirimiphos Methyl	69.3	30.0	3464	69.4	29.4	3470	0	4	34	0	26	52	18	24	6
0.06% Pirimiphos Methyl	76.6	29.0	3831	57.2	23.5	2862	-25	30	57	0	64	0	2	38	60
0.003% Deltamethrin	74.3	29.0	3713	75.1	29.8	3756	+1	3	13	0	6	82	10	8	0

Fig.1: Number of C.megacephala adults settling per 12 fish (Trial SSE).



LIPID OXIDATION AND ASSOCIATED BROWNING IN INDONESIAN SALTED DRIED CATFISH (Arius thalassinus)

by

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ABSTRACT

The effective monitoring of very high levels of lipid oxidation in foods cannot be achieved by methods which measure the presence of hydroperoxides or their decomposition products. Such `primary' and `secondary' products may be reduced to negligible concentrations by further reaction with nitrogenous food components to give `tertiary' products and the possibility of browning.

Such a system is illustrated by the brining and sun-drying of fish, sometimes followed by storage of the product at ambient temperatures for several weeks prior to consumption. Salted-dried fish represents a valuable source of nutrients and revenue in S.E. Asia, the product being characterised by browning and sometimes high levels of free fatty acids and free amino acids.

Analysis of Indonesian salted dried catfish (Arius thalassinus) during processing and storage indicate that although peroxide, TBA and anisidine values give an indication of the onset of lipid oxidation, the values become negligible in the consumed product. Conversely, the significant and steady increases in levels of acetic acid soluble colour and fluorescence found indicate that these parameters are realistic indicators of the tertiary products. Losses of PUFA and the expected decrease in iodine value were found during processing but not during storage.

1. INTRODUCTION

It was pointed out in a FAO review on the assessment and prevention of cured fish losses, (FAO, 1981), that harmful compounds such as lipid oxidation products might be present in cured fish, but that little information was available with respect to their levels and whether they may be causing health, especially nutritional, problems.

Cured fish may be prepared by drying, smoking or salting, and combinations of these treatments. The curing process is important in the S.E. Asian region where about 30% of the marine catch is utilised in this way, mainly at small scale processing sites. A common process in S.E. Asia involves the use of a salting tank containing brine in which the fish are soaked, after which they are left to dry in the sun for an appropriate time, usually between 2 and 5 days.

During the drying process, the water activity of the fish decreases to about 0.70 to 0.75. Salted dried fish would be expected to have a shelf life of 4 to 12 weeks at these water activities, with the main types of spoilage including mould growth, beetle infestation and rancidity development (Poulter et al, 1982; Hanson and Esser, 1985). Browning of the dried product is also considered a problem by the processors as it leads to a loss in retail value of the product (Esser et al., 1986).

Most research on lipid oxidation in foodstuffs has been concerned with the relatively low levels of rancidity found in Western foodstuffs. Little research has been carried out into the assessment and implications of the more extensive lipid oxidation found in some tropical foods such as salted dried fish.

The normal mechanism of lipid oxidation in processed flesh foods is autoxidation of unsaturated lipid, but in salted, sun-dried fish, contributions from photo-oxidation may also be important. These processes lead to the generation of several intermediates including hydroperoxides and peroxides as primary products, eventually producing aldehydes and ketones (e.g. malondialdehyde), together with other breakdown products. These secondary products, especially aldehydes, have the off-flavours associated with rancid oils. Additionally in salted dried fish, hydrolytic and fermentation processes may accompany lipid oxidation to produce free fatty acids and free amino acids from the lipid triglycerides (or phospholipids) and fish proteins respectively.

If lipid oxidation occurs in foods such as salted dried fish, then further reactions are possible between (primary and) secondary lipid oxidation products and other, mainly nitrogenous, food constituents e.g. protein, amino acids and phospholipids (mainly cephalin), to give tertiary products and the possibility of browning. Measurements of fluorescence, believed to be derived from interactions between carbonyl compounds and amino acids (Chio and Tappel, 1969; Kikugawa et al., 1981), together with the determination of colour, arising from further polymerisation type reactions of the fluorescent Schiff base type compounds (Smith, 1988) should give some indication of the extent of these tertiary reactions.

Lipid oxidation in salted dried fish may also lead to nutritional damage, including losses of essential fatty acids, fat soluble vitamins and essential amino acids, but little is known regarding the extent of such losses.

These additional reactions further complicate any study of lipid oxidation in fish products which have been subject to salting and sun-drying. The urgency of such studies has, however, been emphasised by recent reports on the toxicity and possible carcinogenicity of lipid oxidation products, in particular: hydroperoxides, malondialdehyde and polymerisation products (Addis et al, 1983; Ames, 1983; Pearson, 1983).

The work reported in this paper was concerned with determining the suitability of standard methods of lipid oxidation for assessing the degree of lipid oxidation in salted dried catfish (Arius thalassinus) during processing and storage. Alternative techniques were also developed and their suitability assessed. Standard methods may determine either the levels of various lipid oxidation products (i.e. peroxide value - hydroperoxides; thiobarbituric acid (TBA) value - malondialdehyde; anisidine value - unsaturated aldehydes) or changes in the original material (i.e. loss of unsaturation of the lipid - using iodine values or gas chromatography of individual fatty acid methyl esters (FAMES)).

2. MATERIALS AND METHODS

Catfish (Arius thalassinus) were purchased unprocessed at the landing site in Cirebon, Indonesia and then processed in Cirebon as described below.

Twelve catfish were graded according to size into 4 `small' (<1kg), 4 `medium' (~1kg) and 4 `large' (>1kg). They were then split, gutted and washed. Three fish were subsequently taken to be analysed as control (unprocessed) fish. These consisted of a `small', `medium' and a `large' fish. The remaining fish were immersed overnight in saturated brine at tropical ambient temperatures (25-30°C). After salting, 3 fish, 1 `small', 1 `medium' and 1 `large' were removed for later analysis at the School of Food Studies, H.C.H.E., Grimsby, U.K., and the other fish were placed on drying trays, constructed of bamboo matting, which were placed outside in the sun. The fish were dried for 2 to 5 days until the fish were approximately 50% of their original weight. A further 3 fish, as above, were removed after this stage for later analysis in Grimsby. The remaining 3 samples were stored at tropical ambient temperature (25-30°C) inside a wooden box in the processing premises at Cirebon, for 10 weeks.

'All samples were frozen and sent by air to Humberside College where they were stored at -60°C until analysed. Prior to analysis, whole fish (i.e. flesh, skin and bones) were sliced and minced while frozen, and the mince was thoroughly mixed before samples were taken.

The proximate composition (% moisture, % salt etc.) of the fish samples were determined according to the methods of Egan et al, (1981).

Standard methods were used for lipid extraction (Bligh and Dyer, 1959, as modified by Hanson and Olley, 1963) and determinations of peroxide value (Lea, 1952), Wij's iodine value (AOAC, 1984) and % free fatty acid (Egan et al., 1981) on the lipid. A modified procedure, involving the use of glacial acetic acid to clarify some solutions, was used for the anisidine value (Egan et al., 1981). Malondialdehyde content was determined directly on the fish sample using a modified TBA distillation method (Tarladgis et al., 1960; Banasihan, 1985). Measurements of acetic acid soluble colour and fluorescence were also taken directly from fish sample extracts (Eshiet, 1984; Fletcher et al, 1973; Smith, 1988).

A modified methylation procedure (IUPAC, 1979) was used to obtain fatty acid methyl esters (FAMES) from the fish lipid. These were separated and analysed using a SP2330 packed column in a Shimadzu GLC-RAI gas chromatograph with Shimadzu GC 1 data processor. Nitrogen was used as the carrier gas with a flow rate of 25 mL/minute, initial temperature 180°C for 5 minutes rising to 230°C at a rate of 4°C a minute. Nonadecanoic acid was used as an internal standard at a concentration of 1 mg/mL. Standard PUFA retention times, hydrogenation of FAMES and comparisons with previous work with the SP2330 column for fish oil FAMES analysis (Ahmad, 1985; Christie, 1982) were used for identification of peaks.

3. RESULTS AND DISCUSSION

The fish were analysed for % moisture, % protein, % lipid and % salt. The results, shown in Table 1 indicate that, as expected, during drying the moisture content decreases - from approximately 74 to 44% with accompanying apparent increases in % salt, lipid and protein. The final moisture content in the stored sample, at 46.7%, was slightly but significantly higher than the dried figure, indicating an absorption of moisture during storage.

Figure 1 shows the changes in peroxide, anisidine, TBA, % free fatty acid and iodine values during salting, drying and storage of catfish (A. thalassinus).

Peroxide values increased to a maximum during the process before falling to zero in the stored product. This result complements the sharp rise and fall in peroxide values found by Eshiet (1984) and Jeong and Park (1983) during processing and storage of salted dried mackerel (Scomber scombrus) mince and sardine (Sardinops melanosticta) respectively. The peroxide value of the fresh (unprocessed) catfish was 43 mEq/kg lipid and as such would be classified as rancid by Connell (1980). This value may have arisen after catching, since the fresh fish may be held at tropical ambient temperatures (25-30^{o}C) before being sold for processing. Overall, it is possible to conclude that peroxide values are unreliable indicators of lipid oxidation since a low value, in isolation, may indicate either limited or extensive lipid oxidation.

The initial increases in TBA and anisidine values, shown in figure 1, show that further oxidation of hydroperoxides (primary lipid oxidation products) to form carbonyl compounds (secondary products) has occurred. These values reach maxima and then decrease in the stored products, indicating that these determinations are also unsuitable measures of advanced lipid oxidation.

The percent free fatty acid (FFA) results shown in Figure 1 demonstrate that extensive hydrolysis of the lipid has occurred upon salting the fish (up to 30% FFA), with a final level of 50% FFA in the stored samples. This reinforces the observation by Lovern (1962) that hydrolysis in salted cod (Gadus morhua) ceased when the lipid contained 50% of free fatty acids.

The fresh catfish had an iodine value of 118 which decreased by 20% during processing and storage. This indicates that some loss of polyunsaturation through lipid oxidation may have occurred. An examination of the fatty acid profiles of the fish during processing and storage gives more detail of changes in the lipid due to oxidation. The fatty acid profiles of catfish lipid at the various stages of processing are compared in Table 2. The data shows an average 28% decrease in the level of polyunsaturated fatty acids, particularly the eicosa and docosa polyenes, upon salting. A more unexpected change in levels of PUFA, particularly 20:4(n-6), 22:4(n-6) and 22:6(n-3), appeared during storage of salted dried catfish in that these fatty acids showed an apparent increase over this stage. Upon statistical investigation, it was found that this was not a real effect but due to sample variation.

Hence, although there was a decrease in levels of some PUFA during salting of catfish, no significant subsequent changes were recorded during drying and storage. Therefore, the determination of changes in the fatty acid profile appears to be unsuitable as a measure of lipid oxidation in this product.

Figure 2 shows the changes in values of acetic acid soluble colour, and fluorescence during salting, drying and storage of catfish. The colour values remained relatively constant during salting and drying but increased during storage. Fluorescence values, however, remained low during salting but thereafter increased. The observed development of fluorescence which precedes the formation of colour can be explained by the interaction of carbonyl compounds with various nitrogenous (amino) compounds to form light coloured fluorescent intermediates, chiefly Schiff bases, which are then transformed to form brown polymeric products (Pokorny, 1981).

Overall, the increases in colour and fluorescence suggest that these could be more reliable indicators of lipid oxidation in salted dried fish than the Standard methods.

4. CONCLUSIONS

1. Peroxide, TBA and anisidine values were found to be unsuitable for determining the degree of lipid oxidation in salted dried catfish during processing and storage. Values of fluorescence and acetic acid soluble colour appeared to be more useful indicators of lipid oxidation in this product.

2. A 30% loss of polyunsaturated fatty acids was apparent during the salting of catfish, but no further significant change was observed during subsequent drying and storage of the product.

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Table 1: Proximate analysis of salted-dried catfish during processing and storage *

Sample code +	Moisture	Salt	Lipid	Protein (by difference)
	% wet weight basis			
UNPROCESSED				
LU	72.69	0.08	4.01	23.2
MU	76.47	0.09	2.20	21.2
SU	73.77	0.07	3.50	22.9
Mean	74.31	0.08	3.24	22.4
(SD)	(1.75)	(0.01)	(0.81)	(1.08)
SALTED				
LS	70.66	5.13	2.12	22.0
MS	69.78	5.10	4.91	20.2
SS	67.92	5.13	4.25	22.7
MEAN	69.45	5.21	3.76	21.6
(SD)	(1.26)	(0.16)	(1.29)	(1.29)
DRIED				
LD	44.16	10.30	6.81	38.7
MD	42.75	9.97	5.56	41.7
SD	43.59	10.47	4.13	41.8
MEAN	43.50	10.25	5.50	40.7
(SD)	(1.08)	(0.31)	(1.19)	(1.76)
STORED				
LT	47.12	11.44	7.45	34.0
MT	47.22	11.76	4.62	36.4
ST	45.81	11.96	4.28	38.0
MEAN	46.72	11.72	5.45	36.1
(SD)	(0.77)	(0.25)	(1.56)	(2.01)

* All values of % moisture, salt and lipid for each sample are means of triplicate determinations.

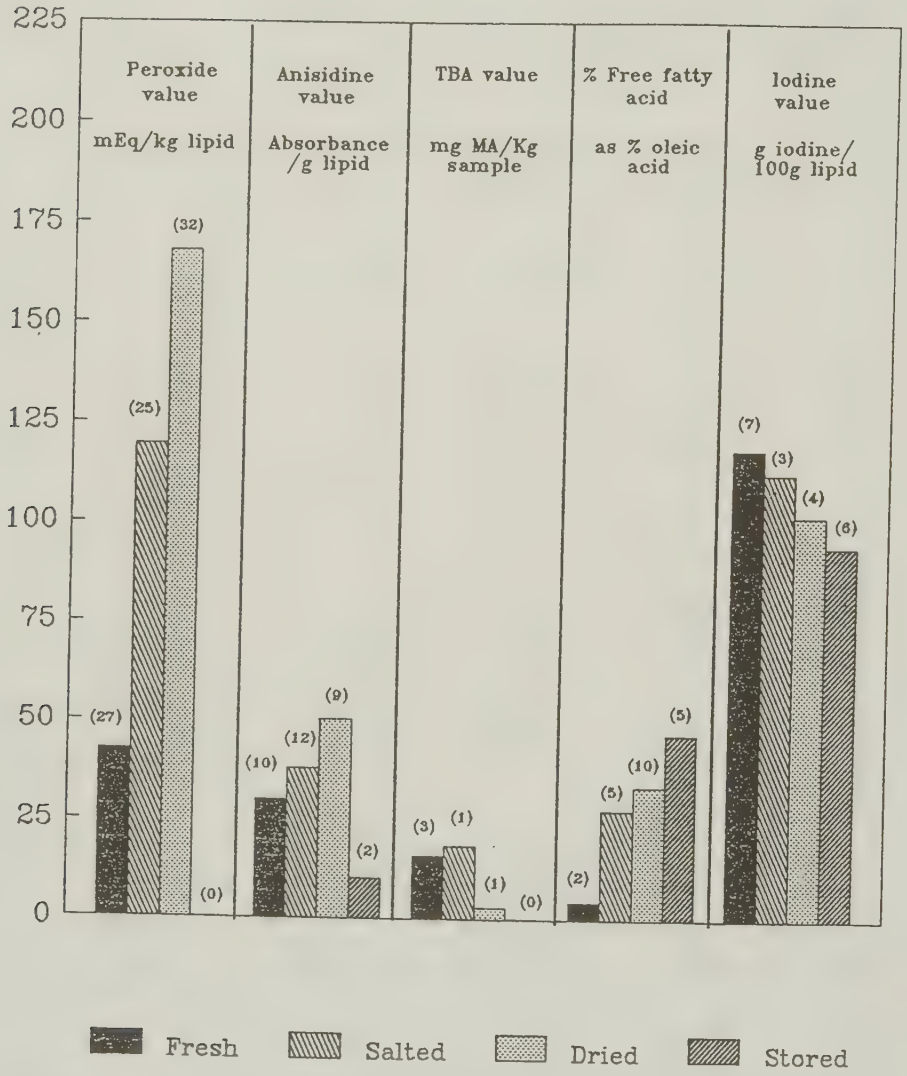
+ The codes L, M and S refer to the large medium and small sized fish samples as defined in materials and methods.

Table 2: The fatty acid profiles of catfish lipid at various stages of processing *

Peak	Unprocessed	Salted	Dried	Stored
14:0	17.7 (1.0)	15.2 (1.4)	16.3 (1.9)	12.9 (0.9)
16:0	230.2 (12.2)	212.7 (32.8)	211.6 (26.3)	193.0 (28.3)
16:1	28.7 (0.5)	26.9 (3.4)	27.6 (1.8)	26.3 (2.8)
17:0	21.3 (2.0)	18.2 (2.9)	18.5 (0.2)	17.3 (2.4)
18:0	90.3 (2.6)	83.8 (11.6)	86.5 (9.0)	83.8 (5.8)
18:1	163.6 (13.1)	151.1 (14.5)	141.9 (19.9)	140.3 (18.7)
18:3	12.4 (0.8)	14.0 (0.6)	11.7 (0.6)	11.5 (0.4)
18:4	3.1 (0.6)	4.1 (1.0)	4.2 (0.7)	4.9 (0.9)
20:4	43.8 (4.2)	30.6 (7.1)	28.0 (3.1)	37.4 (5.3)
20:5	24.3 (2.9)	16.3 (1.1)	14.9 (2.3)	17.1 (3.8)
22:4	14.8 (2.0)	9.4 (1.4)	8.8 (1.6)	14.5 (3.9)
22:5(n-6)	20.1 (1.0)	16.9 (1.9)	17.3 (1.2)	20.9 (3.9)
22:5(n-3)	17.5 (2.1)	12.7 (0.4)	12.0 (2.1)	16.3 (2.6)
22:6	116.0 (7.3)	84.3 (6.4)	79.2 (10.0)	98.0 (7.0)
Unknown	42.0 (7.5)	30.0 (8.1)	28.5 (4.4)	30.0 (6.1)
TOTAL	946 (26)	826 (55)	807 (59)	824 (54)
Calc IV	135.2 (6.7)	123.4 (8.0)	114.3 (2.6)	127.5 (7.7)
Wij's IV	118.4 (11.5)	112.6 (7.9)	101.9 (5.4)	94.3 (8.8)

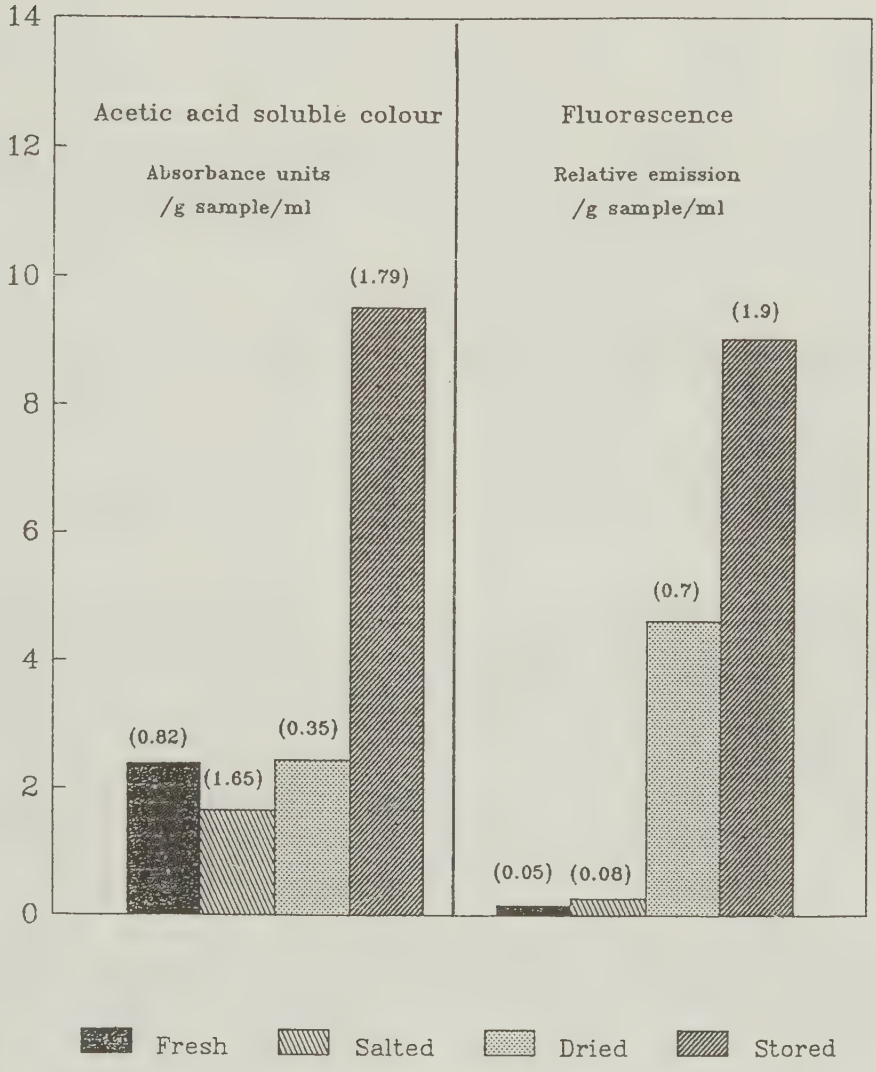
* Average peak areas relative to 19:0 = 100. All values are means of 9 determinations (3 x 3 fish) with standard deviations in parenthesis.

Fig. 1: Lipid oxidation parameters for A. thalassinus during processing



All values are means of triplicate determinations on three fish (ie 9 values, with mean SD in parentheses

Fig. 2: Changes in Colour and Fluorescence during processing



All values are means of triplicate determinations on three fish (ie 9 values) with mean SD(n-1) in parentheses

DRIED SALTED FISH MARKETING AND DISTRIBUTION IN INDONESIA

by

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ABSTRACT

The salting and drying of fish is an ancient method of fish preservation widely practiced in Indonesia. The products are very popular and ranked first among traditional fish products. Almost 40% of the total catch is converted into dried salted products annually. Dried salted fish are marketed locally, inter-provincial and inter-island. Local and inter-provincial marketing is dominated by marketing from outer Java to Java through ports in West Java, Jakarta and East Java. West Java is the biggest market for dried salted fish in Indonesia in which the consumption level is the highest compared to other regions. Although there now exist efforts to export dried salted fish to Asian countries, Europe (Netherlands), USA and Australia; the amount is still negligible. However, the increasing Asian population in Europe, USA and Australia may result in an increased demand for dried salted fish, which in turn could stimulate export of dried salted fish from Indonesia.

Constraints on the dried fish marketing and distribution system in Indonesia are presented and discussed.

1. INTRODUCTION

More than 40% of landed fish is converted into dried salted fish in Indonesia (DGF 1987). This activity involves more than one million small scale fishermen and processors and the methods of processing are very simple and have remained unchanged for years. In spite of this, the marketing and distribution of the products are complicated and face many problems. This paper discusses the constraints on the marketing and distribution system of dried salted fish in Indonesia.

2. DRIED SALTED FISH MARKETING

Dried salted fish producing sites are usually associated with the landing sites. In most cases the consumers are not in the same area. Based on the distance from the consumers and characteristics of the system, the marketing of dried salted fish in Indonesia can be grouped into three categories; local marketing, inter-provincial and inter-island marketing.

In local marketing, the products are distributed or marketed to the surrounding districts or cities. The producers are very much involved in this activity. Since the distance is not very far, problems in packaging and transportation as well as quality are controllable. Quantity of products in local marketing is limited, up to 500 kg per trip. This activity mainly takes place in Java and serves the consumers in the area.

Inter-provincial marketing is defined as the marketing of dried salted fish to consumers in other provinces but still in the same island. Consumer may buy from sales agents or retailers, and the involvement of producers is not as common as that found in local marketing. Marketing activity is mostly done by traders who obtain the products from the producers. This type of marketing is frequently found in Java. Problems in packaging and transportation start to increase, especially in long distance marketing, for example from East to West Java, which usually takes 3 to 4 nights driving. The quantity of the products may be up to 1 long-bed truck per trip (Wibowo, et al. 1985b).

The main producing islands are Sumatra, South Kalimantan and South Sulawesi (Wibowo et al.1985a). Marketing among these islands also exists in much lower volume than those between outer Java to Java's main population centres. The producers do not play any role in inter-island marketing. Dried salted fish from Java is hardly ever marketed to outside Java. This is because the major consumers of dried salted fish are in Java, while Java's production is much lower than the demand.

There now exist efforts to export dried salted fish to Europe, USA, Australia, Asian countries, even Africa. In 1987 4,200 t of dried salted fish from Jakarta was exported to Saudi Arabia. There is also demand for dried salted red snapper in the Netherlands, which Indonesian exporters failed to exploit. In small amounts, dried salted fish is exported regularly to Australia. The increasing Asian population in the USA, Europe and Australia may result in an increased demand for dried salted fish which in turn could stimulate export of dried salted fish from Indonesia. Indonesian exporters are now being encouraged by the Government to increase the dried salted fish export.

3. DISTRIBUTION MAP

The distribution of dried salted fish discussed in this paper mainly covers several provinces such as Riau, South Sumatra, South Kalimantan, South Sulawesi and provinces in Java. The provinces in outer Java are known as the producing centres for dried salted fish in Indonesia. Provinces in metropolitan Java are the main consumers of dried salted fish. The distribution map is as shown in Figure 2.

Dried salted fish from outer Java is mainly exported to metropolitan Java through ports of Merak (West Java), Sunda Kelapa and Jayakarta (Jakarta) and Tanjung Perak (Surabaya) (Wibowo and Putro, 1985). The imported fish from outer Java through Merak and Jakarta are usually from Bangka and Belitung Islands, Palembang, Bagansiapi-api, Riau and Tanjung Karang (all in Sumatra). Dried salted fish from South Kalimantan, on the other hand, are imported through Surabaya, and some through Pasuruan in small quantities. From these cities, dried salted fish are then distributed to other Javanese cities.

The flow of dried salted fish in Java is mainly from east to west, especially the large volume shipments. The majority of dried salted fish from East Java are distributed to Central and West Java through northern cities like Semarang, Cirebon, Jakarta and Bandung. From Jakarta and Bandung they are then distributed further to west Javanese cities (Wibowo et al., 1985b).

Although Central Javanese dried salted fish are mainly distributed to West Java, in small quantities they are also marketed to cities in Central Java and East Java. Similarly West Javanese dried salted fish are mainly distributed to and consumed in West Java, while small quantities are also distributed to Jakarta and cities on the border of West and Central Java (Wibowo et al., 1985b).

4. MARKETING AND DISTRIBUTION CHAIN

Marketing and distribution of dried salted fish involves many institutions or agents making the marketing chain between the producers and consumers long and complex (Hanafiah and Saefudin, 1983). It seems impossible for a newcomer in dried salted fish marketing and distribution activities to start his business while following the system and unwritten rules. In general, the institutions or agents involved in these activities can be broken down into; producer, middleman, wholesaler, inter-island trader, collector and retailer. The links of the institutions or agents in dried salted fish marketing and distribution is shown in Figure 2. The characteristics of each are as follows.

4.1 Producer

Besides processing fresh fish, whether he catches them or obtains them from other fishermen, producers also buy semi-dry fish from other processors to reprocess it together with his fish (Wibowo et al., 1985a). The products are then sold or sent to wholesaler, collector, middleman or inter-island traders. The producers, though, do not have a lot of choice in selling their products. They sometimes have already been given loans from a wholesaler or other institutions to buy and process fish with an obligation to sell the products to them at a predetermined price.

In marketing the products, the producers play an insignificant role except in local marketing. They sell the products direct to the end consumers or retailer in the surrounding area/district.

4.2 Middlemen

Middlemen can be defined as a group of traders not using their own money/capital to fund activities. This group may act on their own behalf or for others. In several areas in outer Java, it is usually wholesaler's or inter-island trader's representatives who are sent to the producing sites to find and order dried salted fish. They may also be residents of the producing area who are paid by wholesaler or inter-island trader. The method of payment is usually based on a commission.

4.3 Collector

This group of traders actively find and buy dried salted fish from producers and resell it to other collectors or retailers. They also supply dried salted fish to wholesalers and inter-island

traders. Also they receive imported dried salted fish from inter-island traders then distribute to other collectors or retailers.

4.4 Wholesaler

Wholesalers trade dried salted fish in big volumes on a regular basis. They obtain fish from collectors or their middlemen. Among wholesalers there exist good linkages, especially information on price, demand and supply in other areas. Trading among wholesalers is also frequent. Supplies for inter-island traders as well as retailers usually come from wholesalers. They sometimes act as inter-island traders as well.

4.5 Inter-island Trader

Dried salted fish export and import among the islands is done by this group of traders. They also supply dried salted fish to wholesalers and vice versa.

4.6 Retailer

This is the last in the chain of dried salted fish marketing and distribution before the fish reach the consumers. Sometimes they only sell dried salted fish in their shops, but in most cases, they sell dried salted fish together with other household needs.

5. CONSTRAINTS IN MARKETING AND DISTRIBUTION

The main technological problems in dried salted fish marketing and distribution are associated with shelf-life, packaging, transportation and storage (Wibowo and Putro, 1985). They shelf-life of traditional dried salted fish is usually short, ranging from 1-2 months. After a few weeks they become mouldy and even spotted by red halophilic bacteria. Packed in wooden boxes the fish also suffer physical damage during transportation and storage leading to spoilage. The conditions of storage are also poor and facilitate insect infestation making the products suffer further loss. To overcome the problems, some wholesalers and inter-island traders reprocess the fish by washing, re-salting as well as drying. Some of them even have cold storage available.

The long marketing and distribution chain also contributes to these losses. The products may reach the consumer 1-3 months after processing. This chain also causes the product to gain a much higher price. However, it is not the producers who enjoy the increase in price, but the wholesalers and inter-island traders instead.

Producers are not able to break the chain by directly exporting the fish to other islands. This is because to directly export the fish to other islands they need strong capital support, which they are lacking. Supplying the fish directly to inter-island traders is also impossible for them, since inter-island traders do not accept fish from producers, or they will accept the product at a very low price.

Fishing seasons also create production problems since they usually coincide with the wet season. Differences in fishing seasons also (see Table 1) stimulate export and import activities. This creates opportunities for inter-island traders to keep the fish and send them to areas where the demand is high due to the off-season.

Efforts should be made to overcome these problems through research . If the government took control of the dried salted fish price and bought them from the processors as they have done for rice and sugar, this would break the chain and improve producer's income.

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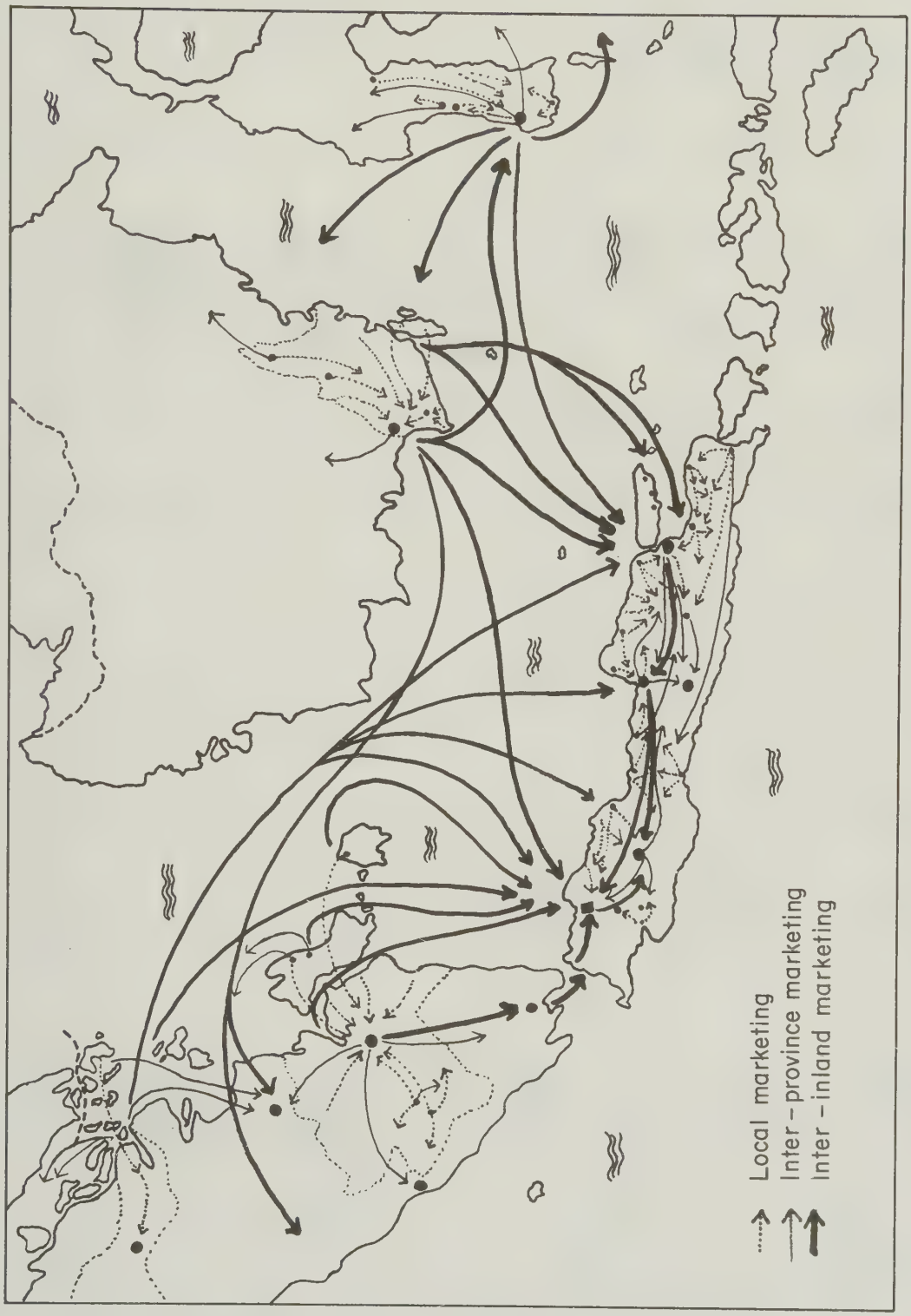

Figure 1 Distribution map of Indonesian dried salted fish

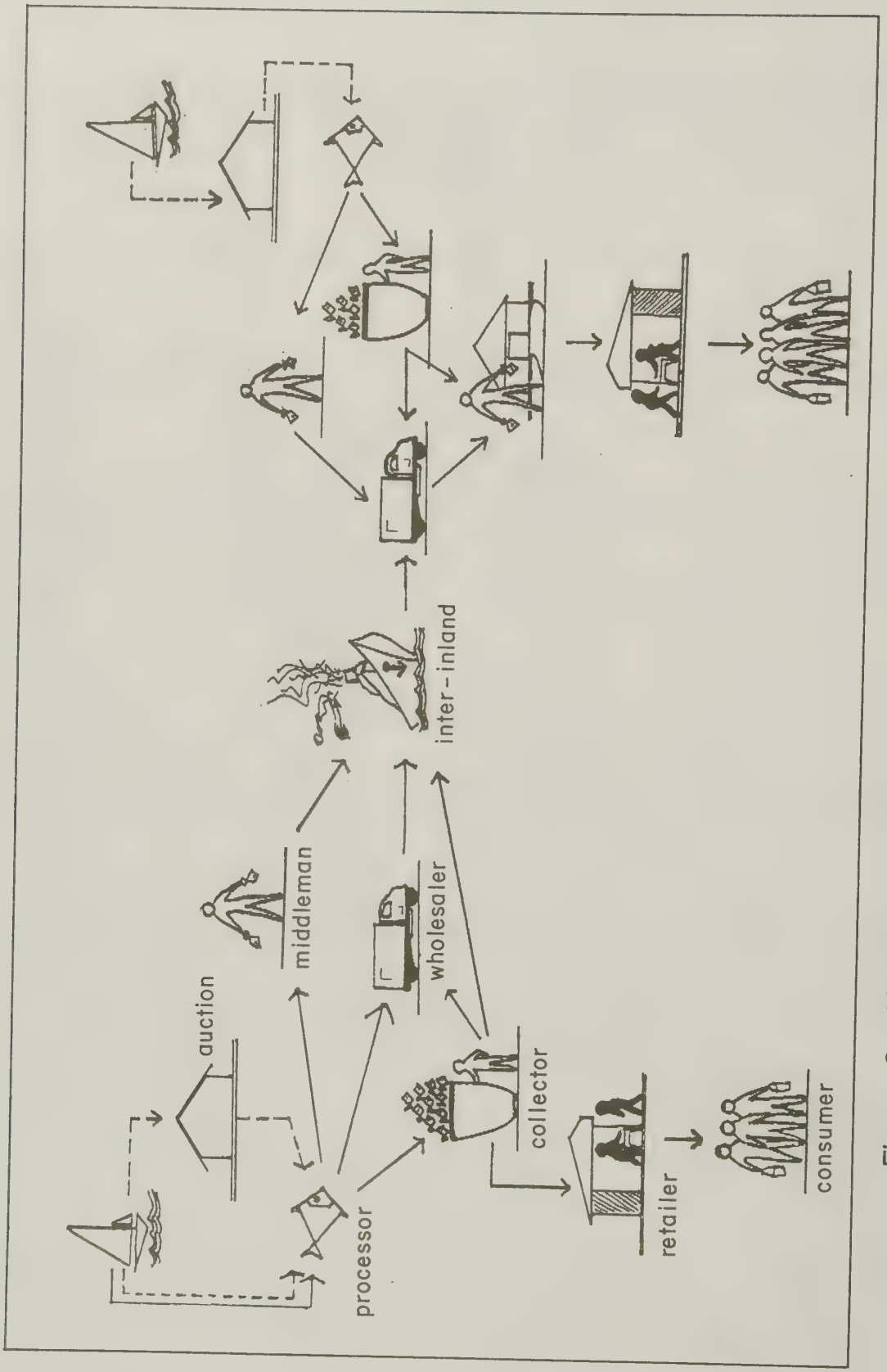

Figure 2 Marketing and distribution chain of dried salted fish

PREMIA AND DISCOUNTS FOR CHARACTERISTICS OF DRIED SALTED FISH

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ABSTRACT

A project jointly funded by the Australian Centre for International Agricultural Research (ACIAR) and the Indonesian Government commenced in 1984 and included an economic appraisal of costs and benefits from improved salting and drying of fish in Indonesia. One segment of this study involved estimation of the components of retail price attributable to product characteristics such as the salt content, the level of water activity and packaging.

Preliminary analysis of a small survey of sales in four markets in Jakarta suggests that, at the means of the variables, water activity, and salt content, salt imposes a discount of approximately 1,800 Rp/kg of dried fish and packaging in plastic attracted a mean premium of about 2,200 Rp/kg of dried fish.

Further research and analysis is directed to improvements in the data and more sophisticated modeling of the complex of characteristics which account for retail prices of dried salted fish.

1. INTRODUCTION

Basic economic theory is goods or product oriented and has only lately taken account of the composition, characteristics or quality of goods. According to the theory the physical properties of goods are presumed to have been incorporated in a consumer's preference map and the theory provides no guide to effects on demand of a specified change in the properties of a good.

Technical change is often directed at changing the quality of products and applied scientific work proceeds from understanding the properties of the product and identification of those relationships which determine the properties of the good and then seeks practical means of modifying the characteristics of a product so that it more nearly matches some `desired' goal.

Product development takes place by the addition or removal of characteristics or by an alteration of the mix of characteristics. The analysis of the prices of products should properly take account of characteristics in the product if we are to understand price formation or identify sustainable prices in a retail market where quality is important.

2. SOME ECONOMIC BACKGROUND

Markets seldom, if ever, reflect basic economic theory which views them as having a simple stable structure - competitive or monopolistic - and trading in homogeneous products. Rather, markets are replete with varying structures, in which price takers become price makers and the reverse and sporadically engage in price discrimination, product differentiation and amalgams of both. The price and non-price offers of retail firms are as complex as the variety of products they sell, the market segments they face, and the composition of each product in their range.

Product differentiation and price discrimination which it makes possible are not, however, engines of market inefficiency but may be conducive to a product-mix which may not be achievable under competitive conditions unless information is indeed readily available and costless. Our understanding of how a potential improvement may be achieved in the product mix is enhanced when we view products as sets of characteristics as proposed in various ways by Waugh (1928, 1929), Theil (1952), Houthakker (1952), Lancaster (1961, 1966b, 1971) and Stigler and Becker (1977). Product variety reflects the search for a match between the quantity and nature of the characteristics which are imparted to products by entrepreneurs and the quantity and mix of characteristics demanded by consumers. As Ladd (1982) has argued, the idea of `product', `good', or `service' is a basic or primitive concept."

The contribution of research to markets by producers and consumers involve costs to both, and market participants continually seek means of economising on these costs. Institutions such as grading, branding, labelling, promotion and clientele relationships develop to achieve economies and their emergence influences the profit-maximising pricing practices of retailers.

The nature of these institutions enables us to `extend' the specification of product characteristics to the shopping environment and perhaps to include the expected characteristics of consumers as indicators of the product and shop characteristics which consumers prefer.

3. RESULTS

A simple model of additive implicit prices of `extended' product characteristics is developed here and implicit prices are estimated from cross sectional data on retail prices for dried fish in Jakarta.

Data was collected by the Research Institute for Fishery Technology (RIFT) in 1987/88 in four markets in Jakarta, Golden Truly, Gelael, Senin, and Glodok. Characteristics of samples of dried salted fish in these markets were subjectively and objectively assessed and prices recorded. The model developed at LTU was used to estimate implicit prices for salt, type of packaging and water activity in dried fish sampled by RIFT.

As with many empirical papers, cited by Ladd (1982), as using hedonic prices or shadow prices of characteristics reference is made here to the work of Lancaster (1971), Muth (1966) and Stigler and Becker (1977) but, as yet, the nature of the theoretical support provided is tenuous.

The principal application of knowledge about implicit prices is that products may be designed to attract optimal prices by imparting to them optimal mixes of characteristics, Ladd and Martin (1977). By identifying characteristics which are imparted to the product at point of sale, account can be taken of shop and consumer characteristics which have been neglected because they have not been properly viewed as subtle forms of product characteristics. (A contrary view can be found in Griliches (1971).)

Taken in this light, market research and demand analyses become profitable since they are likely to improve production efficiency and consumption efficiency and to reduce business failure which arises from errors in the choice of the characteristics-mix in products. As Ladd (1982) and Ladd and Zober (1977) argue, `if the price of the product exceeds the marginal money value of the product's characteristics, the product is not worth what it costs; and it is not purchased.'

Thus the general implicit price equation was of the form

$$(1) \qquad P_g = f(C_i, G_j, S_k)$$

where P_g are the prices of dried fish and the C_i are objective measurable characteristics. State (subjective) characteristics are (G_j). Shop characteristics (S_k) are all state characteristics.

Ladd and Suvannunt present cogent reasons why this `fish price' function may be best estimated in linear form and the strategy adopted here was to use proportions of the constituents and dummy variables such that their coefficients represented premiums and discounts compared with a base class.

Hence the estimating equation would be of the form

$$P_g = \quad _n + \sum_{i=1}^{n-1} (\quad _i - \quad _n) \quad _i + \sum_{k=1}^{m-1} \quad _k Z_k + ? \; W_g + @_g$$

g = 1.....k (fish prices)
i = 1.....n (measurable characteristics)
k = 1.....m (subjective or state)

where $_i$ are the proportions of the components with a base class omitted. The variables used are defined in Table 1.

Z_k are the qualitative variables associated with both product and shops.

$_i$, $_i$ are implicit price premiums or discounts.

W_g is weight of the fish, and $@_g$ are the random errors associated with OLS estimation of (2).

If one is to consider the estimation of the price function of dried fish to derive the implicit unit prices (per kg) of the components, it is possible to derive implicit price functions for each component in the product.

Suppose that there are two components of dried fish salt and `others' and that the relationship of interest is that between retail price and a single characteristic of the product salt. For this situation the relationship might be written for salt as:

(3) $$P_1/W_1 = a_1 + b_1C$$

where a_1 and b_1 are parameters relating P_1, the price per kg of salt in dried fish, to the characteristic which for simplicity is assumed to be a metric variable, say water activity. Then the relationship for other components of dried fish may be written as:

(4) $$P_2/W_2 = a_2 + b_1C$$

if it is assumed that the coefficient (b_1) of the characteristic variable (C) and W_2 is the weight of `other' in dried fish. When the proportion of the total weight of the dried fish which salt is defined as:

$= W_1/W = W_1/(W_1 + W_2)$ so that $W_2/W = 1 -$. The two price functions can then be written as:

(5) $$\frac{P1}{W} = a_1 + b_1C$$

(6) $$\frac{P2}{(1-)W} = a_2 + b_1C$$

Although P_1 and P_2 are not directly observable data can be obtained on the other variables. If one denotes the price of dried fish per kg as $P/W = P_1/W_1 + P_2/W_2$, by rearrangement and addition of the two equations one obtains:

(7) $$\frac{P}{W} = a_2 + (a_1 - a_2) + b_1C$$

If the assumption that b_1 is the same for both components, is relaxed then defining slope parameters for b_1 for salt and b_2 for `other', one may then derive an alternative estimating equation

(8) $$\frac{P}{W} = a_2 + (a_1 - a_2) + (b_1 - b_2)\,C + b_2C$$

The analysis may be extended to n groups of interest from the point of view of implicit pricing of various components of dried fish including attributes such as packaging so that the set of implicit price functions may be written as:

(9) $$\frac{P1}{W_1} = \frac{P1}{1W} = 1 + \sum_{k=1}^{m-1} {}_{1k}Z_k$$

$$\frac{P2}{W_2} = \frac{P2}{2W} = 2 + \sum_{k=1}^{m-1} {}_{2k}Z_k$$

$$\frac{Pn}{W_n} = \frac{Pn}{\sum_{i=1}^{n-1}(1- \ _i)W} = n + \sum_{k=1}^{m-1} {}_{nk}Z_k$$

Summation of (9) yields.

(10) $$\frac{Pg}{W_g} = n + \sum_{i=1}^{n-1} (\ _i - \ _n) \ _i$$

$$+ \sum_{k=1}^{m-1} {}_{1k}Z_k + \sum_{i=1}^{n-1} \sum_{k=1}^{m-1} (\ _{ik} - \ _{nk}) \ _iZ_k$$

The variables in equation (10) are all directly observable and estimation makes it possible to derive the parameters of the set of non-observable equations in (9) and prices of the constituents of the fish.

In this instance Z replaces C and is a set of m dummy variables for product and market characteristics Z_m defined as the base class. Experiments with this form of the implicit price function have been sufficiently successful to confirm the expectation that both product and shop characteristics are important components of implicit prices.

An example of the implicit price functions which may be estimated by this method and employing the specification in equation (10) may be written, with subscripts omitted, as

(11) $$PS = {}_1 + {}_1AQ + {}_1PL + e$$

where PS is the implicit price of salt, 000 Rp/kg
AW is recorded water activity
PL is a dummy variable indicating the use of plastic in the package (no plastic is the base)
$_1$, $_1$, $_1$ are regression coefficients and the e are stochastic errors.

The equation estimated by OLS to derive the implicit price in (11) was as follows:

(12) $$P_W = \underset{(-2.6)}{-27.3} + \underset{(2.02)}{204.4}\ {}_1 - \underset{(02.21)}{336.2}\ {}_1AW + \underset{(3.4)}{51.7}AW + \underset{(6.1)}{2.2}PL$$

$$R_2 = 0.32\ F4,49 = 6.88$$

where $_1$ is the proportion of salt, P_W is the price of dried fish and the other variables are as described above. Taking the derived equation from (11) and (12) and weighting appropriately as the mean of salt content and water activity the implicit price of salt is -1,795 Rp/kg. A selection of implicit prices of salt for various AW levels is presented in Table 1.

On average salt has a negative effect of about 1,800 Rp/kg on the retail price of dried fish and plastic wrapping provides a mean premium of about 2,200Rp/kg.

As shown in equation (12) the statistical performance of the model was excellent for cross sectional material as judged by the usual statistical tests F, t-values and R_2. We are now convinced of the value of this model and given additional data can extend its usefulness to accommodate other variables of interest.

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FISH NOODLES

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ABSTRACT

A fish noodle preparation was formulated using dried fish mince from Nemipterus sp and Oreochromis mossambicus. Fish-enriched noodles had protein contents ranging from 11.27% to 17.65% compared to 10.30% for plain wheat noodles. Product quality was enhanced by the addition of 7% tapioca flour which gave better elasticity and decreasing the moisture content for a firmer texture and not exceeding 3.0% alkaline water for a bright yellow colour. Flour with 10% protein was most suitable for correct colour and elasticity characteristics. Laboratory sensory tests showed that for fried and boiled noodles with 5% and 10% Nemipterus mince, there were no significant differences in texture, flavour, odour and overall acceptability compared to the control. For noodles with Oreochromis mince, fried noodles with 5% and 10% Oreochromis were not significantly different from the control, except for odour in the 10% sample. For boiled noodles, there were significant differences in texture, odour and overall acceptability.

Results from a large-scale survey showed that noodles with Nemipterus mince were more acceptable.

1. INTRODUCTION

Noodles, like rice, are one of the staple foods of Malaysia. There are many types of noodles, and these include `meehoon', `kuayteow', instant noodles, Cantonese and Hokkien noodles. By far the most widely consumed are the Hokkien noodles. These are wet noodles made from wheat flour. The process involves mixing the flour with water and tapioca flour to form a dough. The dough is then progressively reduced in thickness by successive passes through sheeting rollers with decreasing clearance. The last roller cuts the dough into strips of noodles. The noodles are boiled for about 1 min and then thoroughly rinsed with cold water, and excess water drained off. Oil is sprayed onto the cooked noodles to prevent sticking and also to obtain a smooth texture.

Most of the work done on the enrichment of noodles has been limited to the use of vegetable protein (Kaneko, 1966, Murayama, 1974, Clausi, 1971). Wie, Sidwell and Wiley (1969) supplemented a pasta product with fish protein concentrate.

In this study, dried fish mince from tilipia (Oreochromis mossambicus) (freshwater fish) and threadfin bream (Nemipterus nematophorus) (marine fish) were used for incorporation into Hokkien noodles.

2. MATERIALS AND METHODS

2.1 Preparation of the Dried Fish Mince

A double drum dryer (APV, Mitchell Dryers Ltd, England) was used to dry the fish mince. The mince was fed into the drye revolving at 1.2-2.0 rev/min and a drum spacing of 0.10-0.20 mm. The temperature of the dryer was 125-135°C.

2.2 Preparation of the Fish Noodles

The basic formulation used was 63.0% wheat flour, 7.0% tapioca flour, 1.8% salt, 25.2% water, 3.0% alkaline water and 0.5 mL of egg yellow colour (Bush Boake Allen, England). The dried fish mince was incorporated at 2.5%, 5%, 10%, 15% and 20%. The flowchart for fish noodle production is shown in Figure 1.

2.3 Proximate Composition

Moisture, protein, fat, ash and carbohydrate contents were determined according to Pearson (1973).

2.4 Texture Measurement

Texture was determined using an Instron UTM 1140 texturometer, with a Kramer Shearcell. Drive speed and chart speed were 100 mm/min.

2.5 Water Absorption and Cooking Loss

These were determined as described by Dexter and Matsuo (1979).

2.6 Large-scale Survey

The survey was carried out in a town nearby to the university. Noodles with 5% fish mince were used. A total of about 500 people were given samples to taste and a questionnaire to fill in. Care was taken to include the various ethnic groups, occupations and other factors considered important in influencing acceptability.

2.7 Sensory Evaluation in the Laboratory

Twenty experienced panelists were selected to assess the fish noodles. The noodles were prepared according to two standard recipes, fried taiping style and as curried noodles. The noodles were assessed for texture, flavour, presence of unacceptable odour and overall acceptability using a 9-point hedonic scale.

3. RESULTS

3.1 Proximate Composition

The proximate composition of the fish noodles is shown in Table 1. Although protein content was increased by the addition of fish, texture was adversely affected. A maximum of 10% of the dried mince could be used. Above this level, the noodles lost their elasticity and were not acceptable. Some characteristics of the fish noodles are shown in Table 2.

3.2 Sensory Evaluation in the Laboratory

Results for noodles with threadfin mince is shown in Tables 3 and 4. There were no significant differences between the samples tested, thus demonstrating that noodles with 5% and 10% Nemipterus mince were acceptable.

Results for noodles with tilapia mince are shown in Tables 5 and 6. For the fried noodles, no significant differences were detected, except for odour, detected at the 10% level only. For curried tilapia noodles, there were no significant differences in flavour. However there were significant differences in texture, odour and acceptability.

3.3 Large-scale Survey

Table 7 shows that fried noodles with threadfin mince were well-liked. However, noodles with tilapia mince were not acceptable. For curried noodles, both types of noodles were acceptable. The only significant difference was for texture in telapia noodles (Table 8).

3.4 Multiple Regression

Multiple regression was used to determine the factors which contribute to the acceptability of the fish noodles produced and 11 independent variables were selected which might contribute to the acceptability of the noodles. The predicted equation in the analysis is as follows:

$$Y = 5.759 - 0.894S^{d}_{2} + 5.202S^{a}_{3} + 1.276S^{c}_{4} - 0.328S_{5} - 1.039S^{b}_{6} - 0.386S_{7} - 1.276S^{c}_{8} - 0.709S_{9} - 2.038S^{c}_{10} + 0.571S_{11} + 3.187S^{a}_{12}$$

where Y = Acceptability of fish noodles
S_2 = Race
S_3 = Sex
S_4 = Age
S_5 = Occupation
S_6 = Family size
S_7 = Total household monthly income
S_8 = Frequency of fish consumption
S_9 = Frequency of noodle consumption
S_{10} = Price of noodles
S_{11} = Perception of quality of noodles compared to other pasta product
S_{12} = Eating habit

Multiple R_2 = 0.6249
R^2 = 0.3906
F = 1.748

Significant F = 0.1101

Note: The level of statistical significance indicated by a, b, c and d are 5, 10, 20 and 30% respectively.

From the function developed, the R square is 0.3906, indicating that 39.06% of the variation in the fish noodles acceptability is explained by the previously mentioned 11 factors. To analyse the significant difference of each variable, the calculated variable F of 1.748 is compared with tabular F value. The F table value with 11 degrees of freedom is 1.353 at confidence level of 90%.

Among the variables, sex and eating habits contributed most significantly to the regression equation followed by family size, price of noodles, age and frequency of fish consumption respectively. The remaining factors were found to be insignificant in influencing acceptability of noodles.

4. CONCLUSION

Results indicate that threadfin noodles are acceptable. However, with tilapia noodles, acceptability is only marginal. Further research is being carried out to improve acceptability of Oreochromis in noodles.

5. ACKNOWLEDGEMENT

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Table 1

Proximate Analysis of Fish Noodles

Sample	Moisture %	Ash %	Fat %	Protein %	CHO %
Control	54.50	3.04	2.13	10.30	38.40
5% Oreochromis	52.00	2.87	2.01	12.95	38.00
10% Oreochromis	53.00	2.87	2.01	12.95	38.00
5% Nemipterus	52.10	3.24	1.47	13.86	38.77
10% Nemipterus	53.50	3.44	1.28	15.46	37.12

Table 2

Characteristics of Fish Noodles

Sample	ph	Texture kgf	Cooking Loss		Water (%) Absorption
Control	9.95	8.90	0.53		51.72
5% Oreochromis	9.75	7.70	0.97		49.23
10% Oreochromis	9.65	6.50	1.23		46.48
5% Nemipterus	9.60	7.68	1.10	48.68	
10% Nemipterus	9.77	6.55	1.52	46.99	

Table 3

Mean score of organoleptic quality of fried noodles with Nemipterus mince

Organoleptic Quality	Control	5% Nemipterus	10% Nemipterus
Texture	5.82[a]	5.65[a]	5.648[a]
Flavour	6.06[a]	5.71[a]	5.47[a]
Odour	4.00[a]	4.00[a]	4.12[a]
Overall acceptance	5.76[a]	5.59[a]	5.95[a]

[a] Means in a row followed by a common letter are not significantly different.

Table 4

Mean score of organoleptic quality of curried bolied noodles with Nemipterus mince

Organoleptic Quality	Control	5% Nemipterus	10% Nemipterus
Texture	5.65[a]	5.47[a]	4.88[a]
Flavour	6.06[a]	5.88[a]	5.71[a]
Odour	4.37[a]	4.47[a]	4.41[a]
Overall acceptance	6.29[a]	6.09[a]	5.47[a]

[a] Means in a row followed by a common letter are not significantly different.

Table 5

Mean score of organoleptic quality of fried noodles with Oreochromis mince

Organoleptic Quality	Control	5% Oreochromis	10% Oreochromis
Texture	6.67[a]	6.11[a]	5.78[a]
Flavour	6.11[a]	5.89[a]	5.89[a]
Odour	4.61[a]	4.17[ab]	3.94[b]
Overall acceptance	6.41[a]	5.88[a]	5.59[a]

[a] Means in a row followed by a common letter are not significantly different.

Table 6

Mean score of organoleptic quality of curried bolied noodles with Oreochromis mince

Organoleptic Quality	Control	5% Nemipterus	10% Nemipterus
Texture	6.72[a]	5.33[b]	4.56[b]
Flavour	5.44[a]	5.33[a]	5.50[a]
Odour	4.28[a]	3.89[b]	3.50[b]
Overall acceptance	6.41[a]	5.35[a]	4.65[b]

[a] Means in a row followed by a common letter are not significantly different.

Table 7

Mean score of organoleptic quality of fried noodles

Organoleptic Quality	Nemipterus Noodles	Control	Oreochromis Noodles
Appearance	6.06[a]	6.34[a]	5.24[b]
Taste	6.72[a]	6.24[ab]	5.68[b]
Flavour	6.65[a]	6.48[ab]	5.90[b]
Texture	6.10[a]	5.66[a]	4.52[b]

[a], [b]: Means in a row followed by a common letter are not significantly different.

Table 8

Mean score of organoleptic quality of curried noodles

Organoleptic Quality	Nemipterus Noodles	Control	Oreochromis Noodles
Appearance	6.54[a]	6.92[a]	6.00[a]
Taste	6.62[a]	6.62[a]	6.08[a]
Flavour	6.93[a]	6.72[a]	6.14[a]
Texture	5.85[a]	5.46[a]	4.31[b]

a, b: Means in a row followed by a common letter are not significantly different

CHARACTERISTICS OF FISH HYDROLYSATE FROM TILAPIA (OREOCHROMIS MOSSAMBICUS)

by

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ABSTRACT

To improve the flavour of Oreochromis mossambicus, its proteins were hydrolysed using the enzyme alcalase. An acceptable hydrolysate was obtained after 4.5 h of hydrolysis. This corresponded to a degree of hydrolysis of 12.5%. The hydrolsate was analysed for amino acid and fatty acid content, microbiological status and histamine content.

INTRODUCTION

Tilapia, Oreochromis mossambicus, is quickly becoming one of the most popular species in Asian aquaculture. It eats virtually everything and is a prolific breeder. Its market versatility has earned it the nickname "aquatic chicken".

Fish noodles have been developed from O. mossambicus as described in another paper presented to this IPFC Working Party. Its acceptability, however, is not as good as fish noodles produced from marine species. This is mainly due to its flavour components. Panelists did not like the "muddy" freshwater taste of O. mossambicus and preferred the fishy taste of marine fish.

One of the ways in which the flavour could be improved is by enzymic hydrolysis in which proteins are broken down to produce polypeptides thus improving flavour.

This paper describes the hydrolysis of proteins from O. mossambicus using the enzyme alcalase.

MATERIALS AND METHODS

Raw Materials

Alcalase 0.6 L, a commercial protease in which the active enzyme is subtilisin carlsberg, was a gift from Novo Industri A/S, Denmark. The declared activity was 0.6 Anson units/g. The flesh from O. mossambicus was mechanically deboned, leached twice with two times its volume of water and minced in a bowl cutter (ADE SL18, Germany).

The Hydrolysis Reaction

Enzymic hydrolysis was carried out as in Figure 1, in a 2 L vessel with continuous stirring, and pH was controlled by the addition of 4N NaOH to maintain a pH of 8.0. Degree of hydrolysis (DH) was calculated using the method of Satake et al. (1960).

Drying of the Hydrolysate

The neutralised-soluble hydrolysate was spray dried in a Buchi 190 spray drier at an air inlet temperature of 170°C and outlet temperature of 90°C. The feed rate was 4 L/h.

Analytical Procedures

Analyses for moisture, protein, fat and ash were as described in the AOAC methods (1984). Amino acids were analysed according to Kohler and Potter (1967) using a Technicon Sequential Multisample (TSM) Amino acid analyser and integrated with a spectro-physics (Model 22000-011) integrator. Fatty acids were analysed according to Bligh and Dyer (1959). The fatty acid methyl esters were analysed on a Pye-Unicam Series 204 gas chromatograph, using a SP 2330 column packed with supelco syanopropyl polysiloxane of 100/120 mesh. Microbiological evaluations were carried out according to the ICMSF and histamine content was according to Taylor, Lieber and Leatherwood (1978).

Sensory Evaluation

A panel of 20 experienced panelists were chosen to detect bitterness formation in the hydrolysate.

RESULTS

Degree of Hydrolysis

The optimum reaction time was 5 h for an enzyme : substrate ratio of 2.0%. The Dh was 12.5%.

Sensory Evaluation

Panelists detected bitterness after 5 h hydrolysis. At 4-4.5 h, bitterness was slight but was acceptable (Table 1). Hydrolysates obtained after 4.5 h were used for further analyses.

Proximate Composition

The main advantage of the hydrolysate is the reduced fat content (Table 2). Protein content is slightly higher for the hydrolysate.

Amino Acid Profile

The hydrolysate retained the amino acid profile of O. mossambicus with only minor variations (Table 2). Alanine, arginine, cystine, glutamic acid, glycine, leucine, proline and valine decreased slightly while aspartic acid, histidine, isoleucene, lysine, methionine, phenylalanine, serine, threonine and tyrosine increased. Generally the profile is comparable to that of whole egg except for the sulphur amino acids.

Fatty acid Composition

The lipids of O. mossambicus (Table 4) and its hydrolysate have a high proportion of C16 and C18 fatty acids, characteristic of fresh water fish. Except for C18, all the saturated fatty acids in the hydrolysate increased.

Mono-unsaturated fatty acids also increased. The C18:2 and C18:3 fatty acids could not be detected in the hydrolysate as these are unstable and easily oxidised.

Microbial Examination

Although counts increased, the microbial quality of the hydrolysate was still acceptable after nine months storage at 25°C (Table 5).

Histamine Content

The histamine value (Table 6) increased to 12.04 mg/100 g after nine months of storage, well within acceptable limits (Bayer et al., 1981). A high histamine level would indicate extensive bacterial growth.

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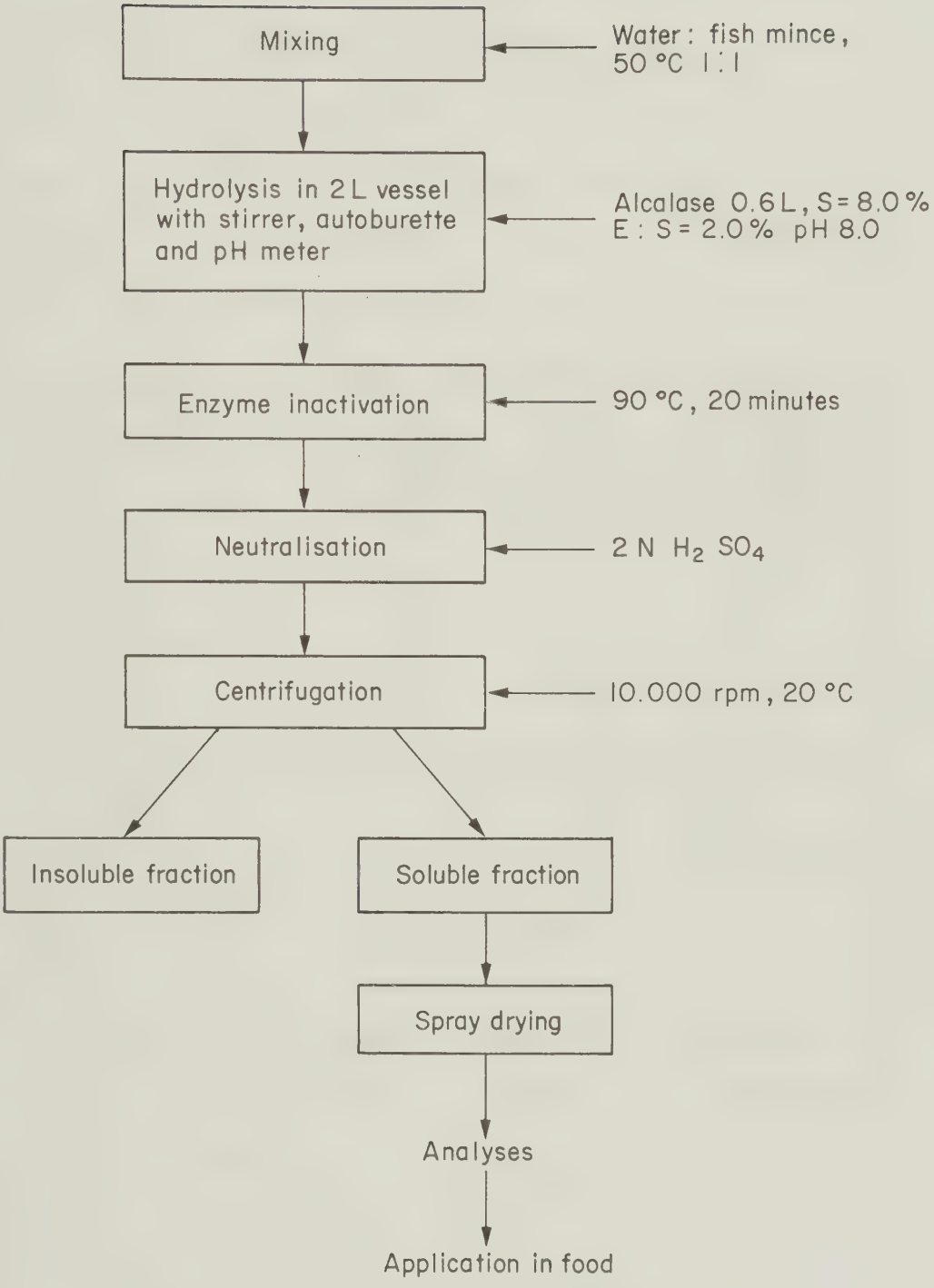

Figure 1 Production of enzymatic fish protein hydrolysate from oreochromis mossambicus

Table 1. Duncan multiple range test for the mean score[1] of bitterness

	Mean score[2]			
	H^1	H^2	H^3	H^4
Bitterness	1c	1.25bc	1.5b	2.25a

1 Significantly different at 5% level
2 Means followed by the same alphabet are not significantly different
H1 Hydrolysate obtained at 3 hours
H2 Hydrolysate obtained at 4 hours
H3 Hydrolysate obtained at 4.5 hours
H4 Hydrolysate obtained at 5 hours

Table 2. Proximate composition of O. mossambicus and its hydrolysate

	Protein (%)	Fat (%)	CHO (%)	Ash (%)
O. mossambicus	80.42	7.42	-	5.16
Hydrolysate	83.07	1.96	1.01	6.16

Table 3. The amino acid composition of O. mossambicus hydrolysate , egg and marine fish hydrolysate

Amino acid	Protein content (mg/g) O.mossambicus	Hydrolysate	Marine fish hydrolysate	Egg
Alanine	64.25	58.17	47.6	56
Arginine*	68.19	60.23	60.2	64
Aspartic acid	86.87	87.37	96.2	91
Cystine	10.93	10.61	6.84	24
Glutamic Acid	148.81	134.81	150.82	124
Glycine	58.03	46.05	29.30	32
Histidine*	26.58	28.61	19.80	26
Isoleucine*	38.04	38.44	45.40	60
Leucine*	73.45	60.89	76.80	87
Lysine*	118.96	127.76	90.5	67
Methionine*	20.48	21.36	32.3	31
Phenylalanine*	30.69	32.22	34.7	56
Proline	26.32	22.63	37.7	40
Serine	34.43	37.24	37.7	72
Threonine*	36.98	37.87	45	48
Tyrosine	24.53	27.89	36.9	38
Valine*	47.76	36.49	44.4	70
Total essential amino acids	461.13	443.87	449.6	509.0
	(50.38%)	(51.10%)	(50.34%)	(51.62%)
Total amino acid	915.30	868.64	892.76	986.0

1 Each value is a mean of 4 replicates
* Essential amino acids

Table 4. Fatty acid composition of O. mossambicus and its hydrolysate

Fatty acids No. of C atom:No. of double bond	Percentage composition O. mossambicus	Hydrolysate
Saturated fatty acid		
C12	0.26	7.72
C13	0.13	0.55
C14	4.53	8.67
C15	1.05	2.86
C16	25.99	37.14
C18	10.08	8.96
C22	9.26	
C24	0.02	
Total saturated fatty acid	52.32	65.90
Monounsaturated fatty acid		
C16:1	8.83	9.80
C18:1	16.56	24.30
Total monounsaturated	25.39	34.10
Polyunsaturated		
C18:2	2.30	
C18:3	10.52	
Total polyunsaturated	12.82	

Table 5. Microbiological examination of the hydrolysate

Time of storage	Viable bacteria/g SPC 25oC	SPC 37oC	Halophiles 37oC	Coliform MPN	Staphylococcus /g
1 day	5.1x105	2.98x105	1320	-	77
4 mths	6.9x105	4.53x105	1900	7	86
6 mths	8.8x105	6.6x105	2280	20	211
9 mths	9.4x105	6.6x105	2330	20	4.8x102

Table 6. Histamine content of O. mossambicus and its hydrolysate

Storage time	O. mossambicus (mg/100g)	Hydrolysate (mg/100g)
1 day	0.29	1.78
4 mths	-	8.98
6 mths	-	11.48
9 mths	-	12.04

THAI FPC

by

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ABSTRACT

Thai FPC is a modification of Fish Protein Concentrate (FPC) type B and is produced from low cost demersal fish resulting in better texture, odour, taste and appearance. The shelf life study demonstrated that the product was still acceptable for a period of one year. The chemical and microbiological results showed that the product was within the standard. Market research has been carried out to determine the nationwide acceptance of the product by Thai consumers, particularly villagers. Tests were also undertaken to determine the proper strategy to promote and to manage the FPC market.

This research has been done by a working committee established by the Thai Fisheries Department, of which representatives from different organisations both government and private sectors participated. This will be the basis for the integration of fisheries products into the Thai National Malnutrition Solving Program.

1. INTRODUCTION

In Thailand, approximately 0.8×10^6 t/year of fish bycatch and surplus low value fish are used for fish meal production. A part of this low value could be utilised for different types of dried products. Thai FPC has been developed to gain the acceptance of low income consumers. This product may be used as a main ingredient in food preparation or as protein supplement. In order to set the integrated distribution of the product, market research and market tests have been carried out.

Fish protein concentrate Type B had been produced for acceptability testing by the Fishery Technological Development Division (FTDD) together with roller-dried fish (RDF) produced by the Institute of Food Research and Product Development (IFRPD). The result of IFRPD acceptability studies were presented and discussed at a FAO/NORAD Round Table Discussion on Fishery Products. Both products were produced from good quality bycatch and were acceptable, becoming "more acceptable" at lower prices. RDF was preferred over the FPC. A suggestion arose at this meeting to investigate a cheaper alternative to the roller-drying process to see if similar product characteristics can be obtained more cheaply (FAO/NORAD, 1982).

2. MATERIALS AND METHODS

The production trials were done mainly at a fish floss factory in Rayong, an eastern coastal province of Thailand. In the deboning trial, the deboning step was done at the Fishery Technological Development Division (FTDD) as was the examination of product quality and the storage stability study.

2.1 Selection of Raw Material

The collection of raw material data was done by observation and the interview of persons at different fish landing places 8-55 km from the factory during 17-26 September 1986. At that stage threadfin bream, barracuda and baby giant mackerel were chosen for the experiment with threadfin bream considered the principle source of raw material. Later, it was found that the price of threadfin bream was higher than the maximum acceptable price during some periods of the year, especially during monsoon season (Apibalpuvanart et al., 1986). Seasonal variations in fish prices was studied using the available secondary data surveyed from 1974-1985 for the fish landing places in Amphur Muang, Rayong Province. The fish species used in this study were wolf-herring, threadfin bream and purple-spotted bigeye. The results showed that the difference in the seasonal price was not significant (Laovapong, 1987).

2.2 Processing Trials

According to Pruthiaranun (1985) FPC type B produced from threadfin bream has a high ash content (22.3%) and low water absorbence. Therefore the fish meat was separated from the bone

before cooking in an attempt to improve the end-product quality. After drying and grinding, the dried products from meat and bone, including offal, are recombined to produce the desirable product.

The first processing trial was to cook the fish meat, bone and offal before drying while the second trial was to dry the meat, bone and offal without cooking (Diagram 1 and 2). Due to the high cost of a meat separating machine, the third trial was performed by modifying the process for fish floss production. The fish were descaled, gutted, cleaned and soaked in brine for 15 min before cooking in a steam cooker. The meat of the cooked fish was separated manually and dried using a steam jacketed drier for 3 h or until the product was of a fine "fluffy" appearance. The residual after meat separation, head and bone, were also dried and ground into a powder, then both dried products were recombined to give a desirable product then cooked and cooled to room temperature before packing (see Diagram 3).

2.3 Analysis and Evaluation

Products from each trial were evaluated as follows:

* Chemical analyses - protein, moisture, fat, NaCl and Ash were determined using AOAC (1980) methods. Water activity was also determined.

* Microbiological analyses - total plate count, Staphylococcus aureus, E. coli, Salmonella, and mould were determined according to the procedure described by ICMSF (1978).

* Water absorbence - water absorbence was measured using a 20 g sample placed into a 100 mL cylinder and observing the volume (a). Water was added up to 100 mL and the mixture was stirred thoroughly and left until no more absorption occurred. The final volume of wet sample (b) was measured to calculate the water absorbence as follows:

$$\text{mL of water absorbed/100 g} = \frac{(b-a)}{w} \times 100$$

* Yield - the weight of fish was recorded during the process from initial weight to the finished product to calculate yield

* Acceptability test - product from each trial was incorporated into a Thai recipe with controlled ingredients. A hedonic scoring method was designed for use in this experiment to elicit the spontaneous opinions of nine to ten panelists on acceptability of the products. A 5-point scale was used with 1 designating the lowest acceptability and 5 having the highest acceptability. Analysis of variance was used to assess the results (Larmond, 1977).

2.4 Storage Stability Study

The FPC used for this study was produced from purple spotted bigeye using the same process conditions as the 3rd process trial. The packages of 100 g of FPC in HDPE bags were prepared for the 1 year study (30±2°C, RH 57-80%). Chemical and microbiological analyses and sensory evaluation of samples were carried out periodically during the storage.

Table 1

A sensory scoring sheet used in the experiment

Items	Samples
Colour	
Odour	
Flavour	
Texture	
Comments:	

3. RESULTS

3.1 Production Trials and Storage Study

The proximate composition, microbiological quality and production yield of FPC from each experiment is shown in Table 2. The moisture content of the fish flesh FPC from all trials was higher than the Thai Industrial Standard Institute (TISI) standard as shown in Table 3, and the total plate counts of the 1st and 2nd trial were also higher than the standard while the product from the 3rd trial was within the set standard. There were no pathogenic bacteria found.

The results of the sensory evaluation and water absorbence revealed that the recombined product from the 1st and 2nd trial had a low water absorbence, unpleasant odour and poor texture due to the inclusion of bone and offal. The 3rd process was produced with a decreased ratio of dried ground bone to meat to improve the texture and water absorbence of the final product.

The results in Table 4 show the proximate composition and production yield of FPC from each experimental trial calculated on the basis of 8% moisture content. It was determined that the production yield as the weight ratio of raw whole fish to recombined FPC were 3.85:1, 3.69:1 and 7.93:1, respectively and the estimated composition of FPC for each mixture is also shown in Table 4.

The composition of FPC during the storage study are presented in Table 5. The results were within the TISI standard, except the moisture content (11.61%) was higher than the standard. However, the water activity of the product was in the range of 0.64-0.70 which was sufficient to inhibit the growth of most moulds (FAO, 1981).

The results of chemical analysis showed no significant change of moisture, protein, fat, ash and NaCl content during storage study. The development of rancidity of the product during storage was significant ($P<0.05$), however the TBA values were low (0.19-0.47 mg malonaldehyde/kg) compared to the values for freshly prepared fish meal (20 to 300 mg malonaldehyde/kg) which was badly oxidized (DFO, 1979).

The sensory evaluation of FPC during storage (Table 6) indicated that odour, flavour and texture was acceptable throughout one year of storage, while the colour of the product showed a significant difference ($P<0.05$), due to the browning of the product. However, the colour sensory score improved when the colour became yellow.

The microbiological assessments of FPC in Table 7 showed that the products were still accepted according to the TISI standard and no pathogenic bacteria were detected during the storage study.

3.2 Cost of Production

The purpose of this study is to estimate the preliminar price of FPC based on a production capacity of about 6,000 kg per month.

It was found that the total cost of production of FPC was 413,078 Baht. This is comprised of total fixed cost and total variable cost. The former was 31,927 Baht or 7.6 percent of total cost while the latter was 389,951 Baht or 92.4 percent. The composition of the total variable cost was about 652.6 percent of the fish input, so changing the input will effect the FPC price by a similar magnitude, that is an increase in fish input cost will lead to the increase in FPC price and vice versa.

From the break-even point method, it was found that if price of fish was 5.50 Baht the FPC price would be 68.85 Baht (Table 8).

3.3 Marketing Studies

3.3.1 Consumers and market research

The market and consumer response to FPC was researched by the Research and Evaluation Division (RED) of the Population and Community Development Association (PDA) in conjunction with the Rural Small Scale Industry's Association (Ruewiwat, 1987). This study assessed the target groups according to income and nutritional criteria. The Parn and Mae Chan districts in the North of Thailand and Nangrong and Putthaisong in the Northeast were selected for the study which was undertaken from October to December 1986.

Cluster sampling was used and each cluster was divided into two groups. The first group had previously eaten FPC while the second group consisted of people who hadn't consumed FPC. The first group was from Parn and Nangrong districts and totally 124 persons while the second group were from Mae Chan and Phutthaisong districts representing 100 persons. Cooking demonstrations were organized for people who had no experience with FPC.

The results of the study revealed that consumers were used to homemade fish powder rather than ready-made fish powder, therefore the promotion of ready-made fish powder should stress the convenience to consumers, such as using FPC as an ingredient in chilli past or with rice and other common Thai dishes. Price was shown to be very important to consumers in deciding whether to purchase FPC. For promotion, it was found that most of the consumers preferred to see television, followed by listening to radio and finally reading newspapers.

3.3.2 Market test

In the Northeast region 60 shops in a 2 month period were surveyed; the average daily sale was 62 units of FPC or 1,582.5 g in the first month and increased to 107 units of 3,036.8 g in the second month. It was noticed that the 35 g pack was preferred more than the 20 g pack. The estimated population of this region was 43,472. Most of the 300 consumers interviewed knew FPC very well and bought FPC at least once a week (The Population and Community Development Association (PDA), 1987).

4. DISCUSSION AND CONCLUSION

Thai FPC is an appropriate product for low income groups because it falls between RDF and FPC type B in terms of quality and cost of production. The beater-drying technique results in a fluffy texture causing improved rehydration. The separation of the steam-cooked fish to dry meat and bone point for further research to save energy and reduce processing times and costs.

Product was distributed to the pilot rural areas in which the majority of the households earn less than 20,000 Baht per year (Ruewiwat, 1987). The highest percentage of those willing to use FPC, falls into the group of households who have household income of less than 5,000 Baht to 10,000 Baht per year.

The Population and Community Development Association Management determined that the FPC could sustain sales of 1886 units per month for an estimated population of 40,000 (through 60 shops) without any special campaign. If the product was placed for a longer period with more experienced distributors, it is expected that the consumption rate would be much higher. A product campaign together with cooking demonstrations and the health benefit of FPC could be considered as part of the product promotion.

Small scale industry is the main target group for the production of the FPC while the target consumers would be in the low protein diet areas probably a long way from production sites therefore distribution costs are of concern. The National Socioeconomic Development has placed a high priority on investment in local fish protein productivity. Currently low quality fish are converted to fish meal for animal consumption which is of low unit value. This situation could be reversed by producing FPC instead. Thai FPC is a long shelf life product, easy and convenient to use in Thai dishes and could be used as a dietary supplement to boost the protein intake of Thai people.

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Table 2

Analysis of FPC (threadfin bream) from the experiment trials

Analysis item	1st meat	1st bone & offal	2nd meat	2nd bone & offal	3rd recombined FPC*1
1. Chemical analysis					
Moisture content (% by wt)	41.45	4.5	31.22	7.36	13.03
Protein content (% by wt)	50.80	52.93	59.72	55.42	68.17
Fat content (% by wt)	4.75	9.12	5.79	10.41	8.96
Ash content (% by wt)	2.92	33.45	3.29	26.81	8.00
Water absorbence (mL H_2O/100g sample)	50	0.0	80	4.5	49.2
2. Microbiological analysis					
Total plate count (colonies/g)	10^8	$1x10^4$	$8.5x10^6$	$6x10^3$	$2.2x10^5$
Staphylococcus aureus	nil	nil	nil	nil	nil
E. coli	nil	nil	nil	nil	nil
Salmonella	nil	nil	nil	nil	nil
3. Production yield					
Weight ratio of FPC to raw material use	1:6.78	1:6.25	1:6.48	1:6.52	1:7.5*2

Note: *1 The recombination ratio of dried fish meat to bone was 3:1

*2 After recombining dried fish meat and bone, there was 1 part of dried ground bone left

Table 3

Thai industrial standard for FPC (TIS 700-2530)

Item	Requirements
Protein (Nx6.25)(percent of dried weight)	min 65
Ash (percent of dried weight)	max 23
Moisture (percent by weight)	max 8
Fluorine (mg/kg)	max 250
Total viable count (colonies/g)	max $1x10^5$
E. coli (MPN/g)	max 3
Staphylococcus aureus	not detected in 1 g
Salmonella	not detected in 25 g
Cl. perfringens	not detected in 1 g
Mould (colonies/g)	max 100

Table 4

Estimates composition and production yield of recombined FPC (threadfin bream) on the basis of 8% moisture content

Item	1st trial	2nd trial	3rd trial
Protein content (% by dry wt)	68.01	66.44	78.38
Ash content (% by dry wt)	22.96	22.99	8.69
Fat content (% by wt)	7.64	9.70	9.48
Weight ratio of FPC to raw material used	1:3.85	1:3.69	1:7.93

Note: The recombination of dried fish meat to bone for 1st, 2nd and 3rd trial were 1:1.5, 1:3 and 3:1 respectively

After recombining dried fish meat and bone, there was 1 part of dried ground bone left on the 3rd trial

Table 5

Chemical analysis of FPC (purple spotted bigeye) during storage
(March 1987 - February 1988)

Time Month	Moisture	Protein	Ash	Fat	TBA no	Water activity	Water absorb.
1	11.61	75.78	8.54	5.11	0.47	0.64	120
2	10.20	-	-	-	0.19	0.67	-
3	10.86	72.09	8.01	4.34	0.19	0.63	108
4	10.05	-	-	-	0.24	0.67	-
5	11.37	74.94	8.44	5.40	0.43	0.70	100
6	10.81	-	-	-	0.42	0.70	-
7	11.52	75.67	8.41	4.96	0.46	0.63	104
8	10.97	-	-	-	0.27	0.64	-
9	10.85	72.78	8.53	4.62	0.31	0.68	105
10	11.27	-	-	-	0.26	0.69	-
11	10.48	74.12	8.44	4.85	0.35	0.64	95
12	10.74	-	-	-	0.30	0.65	-

Table 6

Sensory evaluation of FPC (purple spotted bigeye) during storage
(March. 87 - February 88)

Time (mths)	Colour 5 pts	Odour 5 pts	Flavour 5 pts	Texture 5 pts	Total score 20 pts
2	3.13	3.56	3.50	3.50	13.68
3	3.38	4.00	3.81	3.31	14.38
5	3.50	3.63	3.63	3.25	14.00
8	3.88	3.75	3.94	3.63	15.18
12	4.63	4.06	4.00	3.75	16.44

Table 7

Microbiological analysis of FPC (purple spotted bigeye) during storage (March 87 - February 88)

Time (mths)	TPC (colonies/g)	Mold (colonies/g)	Remarks
1	$2.2x10^4$	0	
2	$8.0x10^2$	0	
3	$5.0x10^2$	5	During the
4	$6.5x10^2$	35	storage
5	$3.5x10^2$	0	*Staphylococcus*
6	$1.4x10^2$	15	*aureus*, *E. coli*
7	$1.3x10^2$	15	*Salmonella*
8	$3.2x10^3$	0	were not
9	$1.3x10^2$	0	detected in
10	$1.7x10^2$	20	products
11	$5.0x10^2$	0	
12	$1.0x10^3$	20	

Table 8

Cost of production of fish protein concentration at 6,000 kgs/month

FIXED COST	%	Baht.
Maintenance	0.43	1,815.00
Rent (Off., Fac., Equip.)	2.37	10,000.00
Salaries	2.25	9,500.00
Prod. Anal/Ins.Fees.	0.36	1,500.00
Depreciation	0.59	2,500.00
Miscellaneous	1.57	6,612.00
TOTAL FIXED COST	7.57	31,927.00
VARIABLE COST		
Input (fish:FPC=8.1), (@5 .50)	62.58	264,000.00
Transport of input (@0.05)	0.57	2,400.00
Labour (30*45*25)	8.00	33,750.00
Energy: firewood	1.42	6,000.00
: electricity	0.71	3,000.00
Packaging	17.57	74,111.00
Storage packet (@0.06)	0.17	720.00
Delivery (@1.00)	1.42	5,970.00
TOTAL VARIABLE COST	92.43	389,951.00
TOTAL COST	100.00	421,878.00
INCOME BY PRODUCT		8,800.00
TOTAL COST (TC)		413,078.00
BREAK-EVEN AT PROFIT = 0 (TR=TC) = P*Q=TC		
	P*6000 =	413,078.00
	P =	68.85

AT FISH PRICE = 5.00 BATH P = 64.84633
AT FISH PRICE = 6.00 BATH P = 72.84633

Diagram 1. The 1st trial of FPC processing

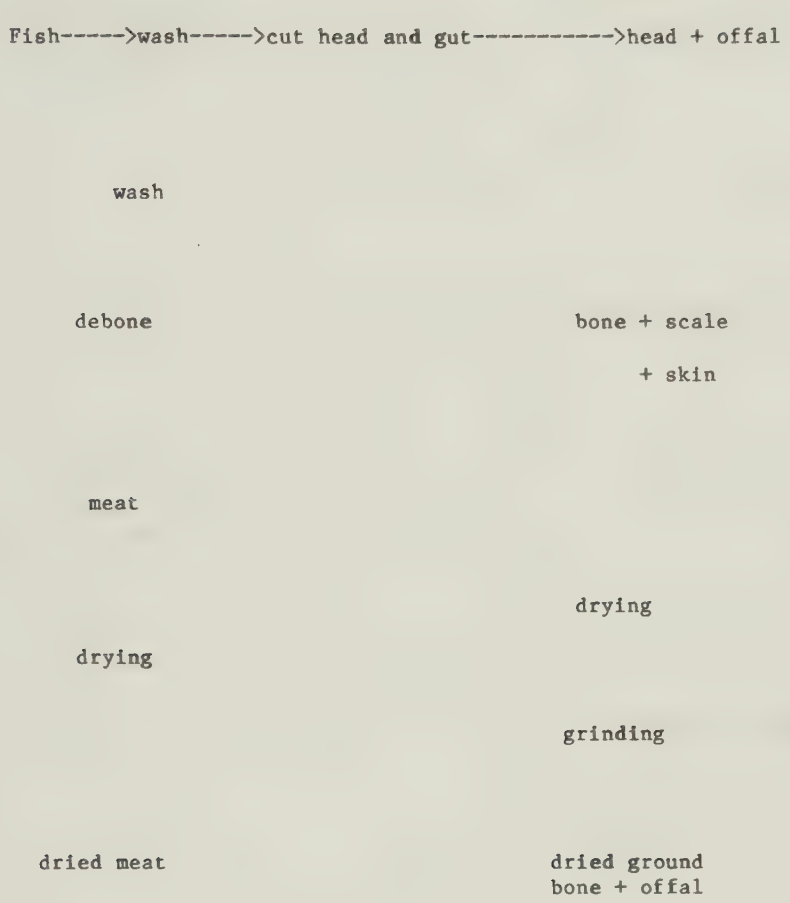

Diagram 2. The 2nd trial of FPC processing

Fish---->wash---->gutted and scaled------>offal--->swill for feed

wash

soak in brine 15 min

rinse with water

steam cooking

debone manually — head + bone + skin

meat

drying — drying

dried meat — dried ground bone

recombine 3:1

FPC

Diagram 3. The 3rd trial of FPC processing

Annex 1

Thai FPC Working Committee

Since there is no proper plant for fish powder production for human consumption in Thailand, it is needed to have a working group to facilitate the development of the processing and the marketing of the production of Fish Protein Concentrate. Thus, the order has been given to the representatives of the departments involved as follows:

1. Ms. Rerngrudee Pruthiarenun, Fisheries Dept., Chairman
2. Ms Charuvan Charoenphol, Health Dept.
3. Ms Theothong Bounyavong, Teacher Educ. Dept.
4. Ms Linchee Sooksuchok, Education Office, Bangkok
5. Ms Siriyong Ruewiwat, Population and Comm.Dev.Ass.
6. Mr Danai Ratananantavatee, Fisheries Assoc. of Thailand
7. Mr.V. Ruengwan Vorawan, V.A.J. Enterprise
8. Mr Avut Apibalpuvanart, Seashine Co.
9. Ms Amporn Lawapong, Fisheries Dept.
10. Ms Supaporn Reungmaneepaitoon, Thai Industry Std.Inst., Secretary
11. Ms Pantip Suwansakornkul, Fisheries Dept, Ass., Secretary

Established on 21 January 1986 by the Department of Fisheries, Ministry of Agriculture and Co-operative, Thailand.

PROCESSED SUPPLEMENTARY FOOD FROM FISH POWDER

by

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ABSTRACT

Nine formulae of processed supplementary foods were prepared and fed to 421 pre-school and school children in Bangkok and the southern part of Thailand and the sensory acceptability of the products was good. As for the storage stability of the products, the recommended duration of shelf life was about 2 months without changes in flavour caused by the hot, humid conditions in Thailand.

Market trials for processed supplementary food were done in 25 primary schools in Bangkok for a three-week period and the results are presented in the paper. Processed supplementary foods are expected to be produced at both village and industrial level in order to solve malnutrition problems.

INTRODUCTION

Protein-energy malnutrition still remains one of the major nutritional disorders of the world. Not only is one half of the population of Africa and Asia suffering from malnutrition, but one half of the child deaths are believed to be due to ignorance relating to the feeding of children.

Thailand established a National Nutritional Policy under the 4th National Economic and Social Development Plan (1977-1981) but the results at the end of the 4th Plan in 1981 were found to be unsatisfactory. In 1981, a study was carried out to supply information to the Food and Nutrition Planning Sub-Committee under the National Food and Nutrition Committee for the Planning of Food and Nutrition in the 5th National Economic and Social Development Plan (1982-1986). After surveys and reviews, protein energy malnutrition was found to be the major problem at the national level. It is necessary to continue in the 6th National Economic and Social Development Plan (1987-1991) the projects in the last plan but with more emphasis on quality and an increased number of target groups and areas. Development of supplementary foods is one of the projects to solve protein energy malnutrition problems which started during the last Plan but supplementary foods also have problems due to many factors such as food habits and time consuming preparation. Processed supplementary foods from fish powder may be able to play an important role in solving these problems. These supplementary foods are made from fish powder because fish is a good source of protein, containing 17-20% protein. Fish protein is a good source of all essential amino acids, the PER of the fish protein ranges from 2.7-3.3 and NPU from 65-70. Fish proteins, being rich in lysine and threonine, effectively supplement cereal proteins.

MATERIALS AND METHODS

Objectives

1. To study processed supplementary food processing practices using locally available raw materials including fish powder.

2. To study the acceptability of processed supplementary food from fish powder.

3. To prepare documents on processed supplementary food derived from fish powder in order to implement in the 6th National Nutritional Policy under the 6th National Economic and Social Development Plan (1987-1990).

Location

1. Laboratory section, Nutrition Division, Department of Health, Ministry of Public Health.

2. Child Nutrition Centres: Bhan Moung-ngam, Amphur Muong, Songkla Province; Bhan Moung-ngam School, Amphur Auong, Songkla Province; Child Nutrition Centre, Bhan Troa Bonn, Amphur Sai Buri, Pattanee Province; Bhan Kalapor School, Troa Bonn District, Amphur Sai Buri, Pattanee Province; Wat Mai Charoenrat School, Nong Chok region, Bangkok metropolitan; Wat Rat Bamroong School, Nong Chok region, Bangkok metropolitan.

Target Groups

1. Pre-schol children, 3-5 years of age

   - Child Nutrition Centre, Bhan Moung-ngam, Amphur Muong, Songkla Province - 49 samples. Each child had 2 acceptability tests for foamulae 2 and 5.

   - Child Nutrition Centre, Bhan Troa Boon, Amphur Sai-Buri, Pattanee Province - 10 samples. Each child had an acceptability test for formula 4.

   - Bhan Kalapoh School, Troa Bonn District, Amphur Sai-Buri, Pattanee Province - 18 samples.

   - Wat Mai Charoenrat School, Nong Chok region, Bangkok metropolitan - 15 samples.

   - Wat Rat Bamroong School, Nong Chok region, Bangkok metropolitan - 20 samples. Each child had an acceptability test for formula 7.

2. School children, above 5 years of age

   Bhan Moung-ngam school, Ampur Muong, Songkla Province - 20 samples.

   - Each standard I child (30 samples) had 2 acceptability tests for formulae 1 and 4.

   - Each standard II child (30 samples) had 2 acceptability tests for formulae 2 and 5.

   - Each standard III child (30 samples) had 2 acceptability tests for formulae 3 and 6.

   Bhan Kalapor School, Troa Born District, Amphur Sai Duri, Pattanee Province, 30 samples.

   - Each standard II child had an acceptability test for formula 1.

   Wat Mai Charoenrat School, Nong Chok Region, 87 samples.

   - Each primary I child (16 samples) had an acceptability test for formula 5.

   - Each primary II child (8 samples) had an acceptability test for formula 4.

   - Each primary III child (18 samples) had an acceptability test for formula 2.

   - Each primary IV child (15 samples) had an acceptability test for formula 9.

   - Each primary V child (12 samples) had an acceptability test for formula 3.

   - Each primary VI child (18 samples) had an acceptability test for formula 8.

   Wat Rat Bamroong School, Nong Chok Region, 102 samples

   - Each primary I child (17 samples) had an acceptability test for formula 5.

   - Each primary II child (20 samples) had an acceptability test for formula 4.

   - Each primary III child (19 samples) had an acceptability test for formula 2.

   - Each primary IV child (20 samples) had an acceptability test for formula 2.

   - Each primary V child (13 samples) had an acceptability test for formula 3.

   - Each primary VI child (13 samples) had an acceptability test for formula 8.

Work Plan

- Search for locally available raw materials for preparing processed supplementary food.

- Select the most convenient processed supplementary food processing practices for use at home level.

- Laboratory test, in cooperation with the laboratory section, in order that both quantity and quality of protein and fat met standards for supplementary food for children set by Public Health Notification No. 87 (B.C. 1985).

- Prepare processed supplementary foods using 9 formulae. The first 3 formulae and formulae 8 and 9 were in chunk form packed into plastic bags and sealed. Formulae 4, 5, 6 and 7 were in powder form packed into coloured paper cone or directly put into cups before eating. Each package contained approximately 20-25 g.

- Select 2 child Nutrition Centres and 2 primary schools in cooperation with the officers from Regional Nutrition Centre No 9, Songkla Province, Songkla Province Public Health and Pattanee Province Public Health. Another 2 primary schools in Bangkok metropolitan area in cooperation with the Education Supervisor from the Supervisory Unit, Bureau of Education, Bangkok.

- Acceptability test in cooperation with the officers from Nutrition Division, Regional Centre No. 9, Songkla Province, Songkla Province Public Health and Pattanee Province Public Health, Child Nutrition Centres, Bhan Moung-ngam and Bhan Troa Bonn, headmasters and teachers from Bhan Moung-ngam school, Bhan Kalapor school, Wat Mai Charoenrat school and Wat Rat Bamroong school, Nong Chok region, Bangkok and the Education Supervisor from the Supervisory Unit, Bureau of Education, Bangkok.

- After distributing processed supplementary foods to the children, collect data by questionnaires, individual interview, group interview and observations.

Supplementary Food Formulae

Formula 1

Ingredients:

Roasted flaked rice	600 g (approximately 10.5 cups)
Fish powder	120 g (approximately 1.75 cups)
Cooking oil	80 g (approximately 0.5 cup + 0.5 tablespoon)
Sugar	200 g (approximately 1.25 cups)
Salt	5 g (approximately 1 tablespoon)

Procedure:

1. Remove impurities from flaked rice, roast well.
2. Make sugar, salt and water into a thickened syrup and then mix the roasted flaked rice, fish powder and cooking oil together with the thickened syrup. Cool on a tray for a period.
3. Pack into plastic bags and seal completely.

Formula 2

Ingredients:

Roasted flaked rice	570 g (approximately 10 cups)
Fish powder	80 g (approximately 1.5 cups + 0.5 tablespoon)
Roasted sesame	150 g (approximately 1.25 cups - 1 tablespoon)
Sugar	200 g (approximately 1.25 cups)
Salt	5 g (approximately 1 tablespoon)
Water	

Procedure:

1. Remove inpurities from flaked rice and roast well.
2. Remove impurities from sesame, wash with water, drain and then roast well.
3. Make sugar, salt and water into a thick syrup and then mix the roasted flaked rice, fish powder and roasted sesame together with the thick syrup. Cool on a tray for a period.
4. Pack in plastic bags and seal completely.

Formula 3

Ingredients:

Roasted flaked rice	600 g (approximately 10.25 cups)
Fish powder	50 g (approximately 0.75 cup)
Roasted peanuts	150 g (approximately 1 cup)
Sugar	200 g (approximately 1.25 cups)
Salt	5 g (approximately 1 tablespoon)
Water	

Procedure:

1. Remove impurities from flaked rice, roast well.
2. Remove impurities from peanuts, wash with water, drain and then roast well.
3. Make sugar, salt and water into a thick syrup and then mix the roasted flaked rice, fish powder and roasted peanuts together with the thick syrup. Cool on a tray for a period.
4. Pack in plastic bags and seal completely.

Formula 4

Ingredients:

Roasted flaked rice	600 g (approximately 10.25 cups)
Fish powder	120 g (approximately 1.3 cups)
Cooking oil	80 g (approximately 0.5 cup + 0.5 tablespoon)
Sugar	200 g (approximately 1.25 cups)
Salt	5 g (approximately 1 tablespoon)

Procedure:

1. Remove impurities from flaked rice, roast well.
2. Mix the roasted flaked rice, fish powder, cooking oil, sugar and salt and then grind to a powder.
3. Pack into plastic bags and seal completely.

Formula 5

Ingredients:

Roasted flaked rice	570 g (approximately 10 cups)
Fish powder	80 g (approximately 1.25 cups + 0.5 tablespoon)
Roasted sesame	250 g. (approximately 1.25 cups + 1 tablespoon)
Sugar	200 g (approximately 1.25 cups)
Salt	5 g (approximately 1 tablespoon)

Procedure:

1. Remove impurities from flaked rice, roast well.
2. Remove impurities from sesame, wash with water, drain and then roast well.
3. Mix the roasted flaked rice, fish powder, roasted sesame, sugar and salt and then grind to a powder.
4. Pack into plastic bags and seal completely.

Formula 6

Ingredients:

Roasted flaked rice	600 g (approximately 10.25 cups)
Fish powder	50 g (approximately 0.75 cup)
Roasted peanuts	150 g (approximately 1 cup)
Sugar	200 g (approximately 1.25 cups)
Salt	5 g (approximately 1 tablespoon)

Procedure:

1. Remove impurities from flaked rice, roast well.
2. Remove impurities from peanuts, wash with water, drain and then roast well.
3. Mix the roasted flaked rice, fish powder, roasted peanuts, sugar and salt and grind to a powder.
4. Pack in plastic bags and seal completely.

Preparation of Raw Materials

Fish powder at home scale level:

1. Large fish

- Remove scales and eviscerate, clean with water
- Steam the whole fish until cooked, discard all the bones
- Cook the fish in a frying pan with medium heat until it becomes a dry powder

2. Small fish

- Clean fish with water
- Steam the whole fish until cooked
- Chop the fish into small pieces
- Cook the fish in a frying pan with medium heat until it becomes a dry powder

Expanded rice at home scale level:

- Remove impurities from rice
- Wash the rice with water and then add water to the rice at approximatley 1.25-1.5 times and then steam for 20 minutes
- Dry the steamed rice
- Fry the rice at a high heat until the rice expands

RESULTS

Results from interviews and observations:

"Supplementary food were very delicious, our school should prepare it for us"
"We preferred to buy supplementary food than other sweets"
"Please let me buy the supplementary food which was left over - I could pay one baht per package"
"This supplementary food was better than the local sweet Kanhom kee Mod because of fatty taste"
"We didn't feel thirsty after eating supplementary food so that we need no water"
"Supplementary food was more interesting than grapes"
"I wanted some more for my brother at home"
"I wanted some more packages because of the taste"
"I prefer to buy supplementary food if it is available instead of biscuits which I used to buy before"
"The school children need no water but some of pre-school children need some water after eating"
"All children could finish eating supplementary food after a short time"
"Such kinds of processed supplementary food are suitable for children"
"We all like it very much"

Distribution of processed supplementary food from fish powder

Some work has been done in cooperation with both the Government and private sector.

1. Department of Health
2. Community Hospital
3. Department of Fisheries
4. Department of Teacher Training
5. Department of Teacher Training
6. Seashine Co. Ltd.

Marketability of Processes Supplementary Food from Fish Powder

At the end of this project it was found that the products were popular but unfotunately not many institutions could produce the fish powder. It was necessary to introduce such products to the private sector for both production and distribution.

Market trials have been done in 25 schools in Bangkok as well as nutrition education for the nutrition teachers. There were 24 schools which accepted the formulae but one school returned some packages due to insufficient time for examination.

It was found that it took some time to introduce the new product initially but once they were accepted, there would be no more problem. Some schools continued to place orders periodically.

Results from market trials

Processed supplementary food from fish powder was found to be good for both children and adults. Texture, flavour and colour are comparable to other sweets but superior nutritive value and lower cost make the supplementary foods much more desirable.

CONCLUSIONS AND RECOMMENDATIONS

1. It is necessary to be friendly to the children when introducing the new product. Good product alone is not sufficient.

2. Even though there were 139 Muslim children out of 197 children, there was no problem in testing because it was mentioned that there was no pork present but in some serious cases the Muslim teachers could play an important role. Local languages were also needed i.e. Southern language suited children of Songkla Province but Yawe language suited children of Pattanee Province.

3. Processed supplementary food from fish powder is only one of several ways to solve malnutrition problems.

4. There are many beneficial factors concerning eating of the supplementary food especially health. Some formula (powder form) suited children with toothache.

5. Cup and spoon are needed for small children but older children could accept any kind of packaging which has been mentioned before.

6. Teachers can play very important roles in selecting formula and packages of supplementary food.

7. The grinder must be cleaned properly and dried before using.

8. All imporities i.e. bone, stone, rice husk etc. must be removed before processing.

9. The malnourished children neec processed supplementary food. Approximately 50 g per day in addition to the ordinary meals, is suitable.

10. Processed supplementary food from fish powder can be kept for two months in a cool and dry place.

The private sector could play a very important role in solving malnutrition problems. The processed supplementary foods made from fish powder were found to be acceptable to consumers and could compete with other supplementary foods. It should therefore be introduced commercially in order to meet the requirements of people in both urban and rural areas.

ACKNOWLEDGEMENT

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The authors are very grateful to Mrs. Rerngruedee Pruthiarenun, Chairman of the FPC Working Committee, Department of Fisheries, who contributed her guidance to promote the processed supplementary food from fish powder.

The next two important groups to be acknowledged ar Mr. Banchong Vithayametar, Regional Nutrition Centre No. 9 (southern region) who coordinated each of the institutions in Songkla and Pattanee province and Mrs. Linchee Sooksuchok, Working Member of the FPC Working Committee, Education Office, Bangkok, who coordinated primary schools in Bangkok for acceptability testing.

Last but not least, the authors are very grateful to Mr. Avuth Apibalpuvanart, Seashine Co Ltd, Working Member of the FPC Working Committee who played a very important role in the market trials.

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Table 1

Quantity and quality of protein and fat of processed supplementary food from fish powder (per 100 calories)

Formula	Ingredients	Protein (g)	Amino acid score		Fat (g)	Linoleic acid (mg)	Fatty acids that are longer than 20 carbon atoms (cal)
			Cystine and Methionine	Lysine			
1	Roasted flaked rice:fish powder: oil:sugar:salt (60:12:8:20:0.5)	3.17	100	140	2.66	1418	0.41
2	Roasted flaked rice:fish powder: roasted sesame:sugar:salt (57:8:15:20:0.5)	3.20	111	116	2.46	946	0.71
3	Roasted flaked rice:fish powder: roasted peanuts:sugar:salt (60:5:15:20:0.5)	3.35	97	89	2.22	799	0.28

NB: All formulae met standards for supplementary food for children (in quantity and quality of protein and fat) according to Public Health Notification No. 87.

QUALITY DEVELOPMENT OF DRIED SQUID

by

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ABSTRACT

To overcome the rapid change of dried squid colour, certain chemical reagents namely potassium metabisulphite (KMS), citric acid, lactic acid, potassium sorbate, sodium polymetaphosphate and acetic acid were chosen at various concentrations to soak squid before drying. The results showed that dried squid which were soaked before drying in 0.6% potassium sorbate plus 4.0% sodium polymetaphosphate with pH adjusted to 5.9 by acetic acid gave the best colour. The affect of packaging materials, polyethylene bag, paper bag and vacuum bag were studied along with storage temperature (-18, 5 and 10°C) in order to maintain dried squid quality during storage. The results showed that the type of packaging had no effect on colour but storage time and temperature had an effect on the colour of dried squid. Dried squid which were stored at -18°C were best in colour. Dried squid could be kept at -18, 5 and 10°C for approximately 90, 75 and 45 days respectively with acceptable quality. Proximate and microbiological analysis of dried squid which were soaked before drying in 5% brine and 0.6% potassium sorbate plus 4.0% sodium polymetaphosphate with pH adjusted to 5.9 by acetic acid were carried out during different times of storage (0-90 days at 5°C). There were small changes in moisture, fat and protein (total nitrogen x 6.25) content of dried squid during 90 days storage at 5°C no matter what type of solution was used before drying. The value of moisture, fat and protein of dried squid were in the range of 23.27-25.5%, 3.57-3.93% and 62.92-65.1% respectively. Independent of storage time and type of solution used before drying, microbiological data were in the range as follows: total plate count 2.15×10^3 - 6.3×10^3 cfu/g, mould count 30-185 cfu/g, S. aureus 0-90 cfu/g, coliform 30-240 MPN/g and E. coli 0 cfu/g.

1. INTRODUCTION

In 1983 Thailand produced approximately 132,044 t of cephalopods worth 2.687 million baht, with 26.43% of the total used for dried cephalopod production of which 12% of total production was exported, which is mainly dried squid. Dried cuttlefish and octopus are not as popular as dried squid.

High quality dried squid for export have to be ivory white colour and symmetrical with no damage. It was observed that dried squid at room temperature (28-30°C) change their colour from ivory white to brown within 3-4 days. This colour change is due to non-enzymatic browning reaction, a reaction of amino groups and reducing sugar or carbonyl compounds (caused by fat oxidation) in dried squid. Factors which retard non-enzymatic browning are low temperature storage, low moisture content and some chemicals e.g. potassium metabisulphite (KMS) (Stansby, 1963; Hayashi and Takagi, 1979).

Some researchers believe that using reagents such as potassium sorbate, sodium polymetaphosphate and adjusting the pH to 5.9-6.0 with acetic acid, can retard colour change and mould growth (Sheehy and Vik, 1980). Therefore, it is the purpose of this study to determine the effects of certain chemical reagents used for soaking squid before drying, type of packaging, and storage time and temperature, on colour quality of dried squid. The composition and microbial content of dried squid were also determined.

2. METHODS

2.1 Effect of Chemical Reagents on Dried Squid Colour

Twenty-four hours iced squid were used for drying. The drying method was as follows:

SQUID

Split the tube, eviscerate, remove eyes and beak

Remove coloured skin and wings

Wash

Soak in 5% salt solution (w/w) for 3 mins

Wash

Soak in test solutions for 3 mins

Drying

Dried squid

Squid were soaked in one of the following test reagents:

1.1 Potassium metabisulphite (KMS) 0.1, 0.2% (w/w) adjusted to pH 5.5 with lactic acid.

1.2 Potassium metabisulphite (KMS) 0.1, 0.2% (w/w) adjusted to pH 5.5 with citric acid.

1.3 Potassium sorbate 0.6% plus sodium polymetaphosphate 4.0% adjusted to pH 5.9 with lactic acid.

1.4 Potassium sorbate 0.6% plus sodium polymetaphosphate 4.0% adjusted to pH 5.9 with acetic acid.

Dried squid were kept at 28-30°C for 5 days. Five samples were collected from each treatment at 1, 2, 3, 4, 5 days respectively. Seven trained panelists were used to evaluate colour quality of dried squid by means of a 5 point hedonic scale where 5 equals ivory white colour and 1 equals red brown colour.

2.2 Effect of Type of Packaging on Dried Squid Colour

Sixty dried squid processed in a salt solution only were divided into 3 groups:

Group 1. Packed two squid in each polyethylene bag, with holes for ventilation on both sides of each bag.

Group 2. Packed two squid in each paper bag.

Group 3. Packed two squid in each vacuum bag (polyvinylidene chloride layered with polyethylene) and included a small package of deoxidizer in each bag.

The dried squid were then stored at -14°C where light was turned on 24 h every day. A trained panel was used for evaluating squid colour by comparing with standard colours (Figure 2). Comparison of colour score among three different groups were made and analysed statistically.

2.3 Effect of Storage Time and Temperature on Dried Squid Colour

Dried squid were prepared according to 2.2. The dried squid were packed in either polyethylene bags and multilayer bags (PE/brown paper) then packed together into a corrugated box. The squid were divided into 3 groups:

Group 1. Kept at approximately -18°C
Group 2. Kept at approximately 5°C
Group 3. Kept at approximately 10°C

Five samples were taken from each group at 1, 7, 13, 21, 30, 45, 60 and 90 days storage for colour evaluation by seven trained panelists according to procedure in 2.1. The data were compared for different storage times and temperature.

2.4 Composition and Microbial Content of Dried Squid During Storage at 5oC for 90 Days

Dried squid were prepared according to the procedure in 2.2 and packed in a polyethylene bag then stored at 5°C for 90 days. Four samples were taken for moisture (dried at 102°C for >2 h), fat (ether extraction) and protein (Macro-Kjeldahl method) analyses at 0, 7, 14, 21, 28, 45, 60 and 90 days. Also, three samples were taken for microbial analysis at 0, 7, 13, 21, 35, 45, 56 and 90 days. Total plate count (TPC), mould count, E. coli, coliform and S. aureus were determined according to APHA (1984).

3. RESULTS

See Tables 1-5.

4. CONCLUSION

1. The type of reagent used for soaking squid before drying showed a significant effect ($P<0.05$) on dried squid colour. The most effective reagent was 0.6% potassium sorbate plus 4.0% sodium polymetaphosphate and pH adjusted to 5.9 by acetic acid.

2. The different types of packaging showed no significant effect ($P<0.01$) on dried squid colour. The most suitable packaging system is the polyethylene bag with some holes for ventilation since its cost is cheap and the process is convenient.

3. Dried squid can be stored at -18, 5 and 10°C for 90, 75 and 45 days respectively with acceptable quality.

4. There were small changes in moisture, fat and protein content of dried squid stored at 5°C for 90 days. These values were as follows:

moisture content = 23-25%
fat content = 3.75-3.85%
protein content = 62.92-64.66%

5. Independent of storage time and the type of solution used before drying, microbial data were in the range as follows:

TPC = $2.2\text{-}6.3 \times 10^3$ cfu/g
mould count < 30-185 cfu/g
S. aureus 0-90 cfu/g
Coliform <3-240 MPN/g
E. coli 0 cfu/g

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Table 1 Means color scores of dried squid which were treated by different reagents before drying. Dried squid were kept at 30-32°C for 5 days.

Storage time (days)	color score[1]						
	KMS 0.1 + citric	KMS 0.1 + Lactic	KMS 0.2 + citric	KMS 0.2 + Lactic	Sorbate +PO_4 + Lactic	Sorbate +PO_4 + acetic	Control[2]
1	4.66[a3]	4.76[a]	4.00[b]	4.00[b]	4.79[a]	5.00[a]	4.61[a]
2	4.14[ab]	4.00[ab]	3.54[c]	3.77[bc]	4.00[ab]	4.26[a]	3.60[c]
3	3.29[ab]	3.17[ab]	3.29[ab]	3.39[ab]	3.43[ab]	3.50[a]	3.03[b]
4	3.07[a]	2.90[a]	2.40[bc]	2.43[bc]	2.33[c]	2.71[ab]	2.21[c]
5	2.29[a]	1.57[c]	2.21[ab]	1.86[bc]	1.86[bc]	2.33[a]	1.14[d]

[1]Means color scores from 7 trained panels : 5= ivory white color
1= red brown color

[2] "control" were dried squid which were treated with 5% salt solution

[3] Means Scores in same line with identical letters are not significantly different ($P < 0.05$)

Table 2 Means color scores of dried squid which were stored at -14°C in different type of packaging

storage time (months)	means color scores[1]		
	Polyethylene bag	Paper bag	Vaccuum bag
0	4.54[a2]	4.64[a]	4.56[a]
1	4.26[b]	4.14[b]	4.19[b]
2	4.28[c]	3.98[c]	4.11[c]
3	4.23[d]	3.97[d]	4.04[d]

[1]Means scores from 20 samples ; 5 = ivory white color
1 = red brown color

[2] Means scores in same line with identical letters are not signifiantly different ($P < 0.01$)

Table 3 Means color scores of dried squid which were stored at 10, 5 and (-18)°C for 90 days.

storage time (days)	Means color scores[1]		
	10°C	5°C	-18°C
1	4.75[a2]	4.75[a]	4.75[a]
7	4.57[a]	4.61[a]	4.75[a]
14	4.29[a]	4.61[ab]	4.79[b]
21	4.42[a]	4.64[ab]	4.82[b]
30	4.31[a]	4.43[ab]	4.71[b]
45	3.55[a]	4.26[b]	4.65[b]
60	2.96[a]	4.05[b]	4.65[c]
75	2.75[a]	4.14[b]	4.64[c]
90	2.57[a]	3.46[b]	4.54[c]

[1] Means color scores from 7 trained panels
5 = ivory white color 1 = red brown color

[2] Means scores in same line with identical letters are not significantly different ($P < 0.01$)

Table 4 Chemical composition of dried squid stored at 5°C for 90 days.

stoage time (days)	% moisture	%Fat	%Protein (TNx6.25)
0	25.46	3.65	64.25
7	24.44	3.73	64.08
14	25.55	3.85	63.75
21	23.92	3.73	64.66
28	24.67	3.63	63.97
45	24.62	3.57	62.92
60	23.27	3.58	63.76
90	24.92	3.64	64.18

Table 5. Microbial analysis of dried squid treated with two different reagents and stored at 5°C for 90 days

Storage time (days)	TPC (colony/g)		Mould count (colony/g)		Coliform bact. (MPN/g)		*S. aureus* (colony/g)		*E. coli* (colony/g)	
	Control[1]	Sorbate + Acetic acid	Control	Sorbate + Acetic acid	Control	Sorbate + Acetic acid	Control	Sorbate + Acetic acid	Control	Sorbate + Acetic acid
0	$6.3x10^3$	$5.4x10^3$	80	30	240	240	65	80	0	0
7	$5.6x10^3$	$4.65x10^3$	185	30	240	3	40	90	0	0
14	$3.1x10^3$	$3.3x10^3$	51	30	23	3.6	30	90	0	0
21	$3.3x10^3$	$3.19x10^3$	85	30	3	3	10	60	0	0
35	$2.15x10^3$	$2.31x10^3$	55	45	3	3	10	30	0	0
45	$4.12x10^3$	$4.89x10^3$	30	30	3	3	0	5	0	0
56	$4.44x10^3$	$5.91x10^3$	35	47.5	3.6	3	10	0	0	0
90	$3.53x10^3$	$4.97x10^3$	35	35	3	3	0	0	0	0

[1] Control were dried squid which were treated with only 5% salt solution

THE STORAGE OF VACUUM PACKED, HEAT STERILISED TRENCHED SARDINES (AMBLYGASTER SIRM) AT AMBIENT TEMPERATURES

by

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ABSTRACT

Dressed, brined, trenched sardines (Amblygaster sirm) vacuum packed in transparent retortable nylon-polyolefin pouches (200 g) and sterilised at 212°C for 30 minutes gave a product similar to canned fish with a drained mass of 75.5-77.5%. The total oil content of the product ranged from 1.9-4.9%. A higher concentration of oil was detected in the skin (5.9%) compared to flesh (2.9%). The dominant fatty acids in raw trenched sardines were 16:0 (22.4%), 22:6 (22.2%), 18:1 (12.4&) with toal saturates amounting to 38% and polyunsaturates 39%. No marked changes in the fatty acid profile was observed during vacuum packed, heat sterilisation. The packs were stored at ambient temperature (28±2°C) and observed for a period of 75 days and a change in peroxide value from an initial value of 19.1 milli eq/kg oil to 103.2 milli eq/kg oil after 75 days was noted. However, there was no yellowing or any rancid odour development during the period of storage. Similarly, little change in moisture content (74.3%.-72.5%) and pH (5.9) was noted, indicating that the packs were in an acceptable condition during the storage period of 75 days. Packs processed using 5 h delayed iced raw material showed changes comparable to the above packs. There was a positive correlation between peroxide values of immediately and delayed iced, heat sterilised fish ($r=\pm 0.8222$, ≤ 0.05).

Thus, vacuum packed heat sterilised fish could serve as a means of preserving seasonal gluts of small shore-seine varieties and could also serve as an alternative for canning with a considerable saving on the cost of packaging.

INTRODUCTION

In Sri Lanka in 1984 40% of the marine catch consisted of small shore-seine varieties such as Sardinella spp., Anchoviella sp., Thryssa sp., Amblygaster sirm, Rastrelliger kanagurta, Pellona sp., etc. Even though these varieties have a lower market value compared to the larger varieties, they are very popular, especially in the fresh form among the lower and middle income groups. A vacuum packed, heat sterilised product would provide an alternative method of preservation of the seasonal gluts of these varieties to the freezing or drying that is already practised.

Keay (1982) states that the geometry of the pouch is such that heat transfer is rapid and sterility is achieved with less damage to the intrinsic properties of the food. Chia, Baker & Hotchkiss (1983) state that in rainbow trout, pollock and shrimp, higher sensory scores and better texture and colour were obtained for a pouched product than a canned product, while the pouched products had lower amounts of NH3, TMA and total carbonyls.

Trenched sardines contained moderate levels of oil and thus would be a suitable raw material for retort pouching as an alternative to fresh fish marketing.

Most small shore seine catches are obtained by gill netting or from beach seines, and are generally transported in ice to marketing centres. A previous study (Chinivasagam and Vidanapathirana, 1985) indicated that a delay of up to 5 h in icing did not have any marked effect on the quality of trenched sardines Amblygaster sirm.

This study was carried out with the aim of evaluating the nature and storage characteristics of vacuum packed, heat sterilised trenched sardines which are obtained using local conditions.

MATERIALS AND METHODS

Raw material

Fish were obtained directly on landing from two separate boats a and b (around 6.30 am). A portion of the catch from a and b was washed and iced on landing, while the balance was washed well and stored in a fish box at ambient temperature (26±1°C). Care was taken to cover the fish to prevent dehydration. Size of the fish used in this study ranged from 14-16 cm and consisted of a mixed catch of mature males and females.

Processing and Packaging

The fish were dressed and processed as previously descirbed by Chinivasagam and Etoh (1985). The dressed, brined fish were vacuum sealed and sterilised at 121°C for 25 min and then rapidly cooled using water under pressure.

Nature of the Pouch

The pouch was made of transparent nylon/polyolefin (double layer pouch) with oxygen transmission rates of 55 cc/m^2 day atm. at 27°C and RH 65%. The material was obtained from Toyo Seikan Company, Japan.

Storage

The pouches (processed from lots a, b of immediately iced fish and lost a, b of delayed ice fish) were stored in a dark room at 28±2°C.

Evaluation of Samples

Quality changes of the pouched products were evaluated every two weeks for a period of 75 days. Initially two batches of raw material (a and b) which were subjected to immediate icing (0 h) and delayed icing (5 h) were subjected to chemical analysis and sensory evaluation. The pouched product was also similarly analysed.

Peroxide value and moisture content was analysed according to the methods of the 1977 CIDA/FAO/CECAF training programme on fish handling, plant santiation, quality control and fish inspection. Oil content was analysed according to Bligh and Dyer (1959) for the flesh of raw fish and total contents of the pouched product.

Fatty acid composition was determined by gas liquid chromatography (GLC) of methyl esters. The esters were prepared using BF_3/methanol. GLC analysis was performed on a 6'x88" ss column packed with Gp 10sp 2330 on 100/120 Chromosorb WAW, using a Varian model 3700 GLC and Hewlett Packard 3390 A integrator. The oven temperature was 210°C. Identification of C:16, C:14, C:18 was carried out using standards (Poly Science Corporation) while the rest were identified using cod liver oil as a reference standard (Ackman & Burger, 1965).

RESULTS

The raw trenched sardines (Amblygaster sirm) showed a mean moisture content of 74.9% and 69.8% in immediately iced (0 h) and delayed iced (5 h) respectively. The oil contents of the raw material (0 and 5 h) used in this study ranged from 3.9-6.5% while the pH was 5.9-6.0 respectively (Table 1).

Fatty acid composition in raw immediately iced Amblygaster sirm contained 38.2% saturates which was dominated by palmitic acid (16:0) followed by stearic acid (18:0) while the major monounsaturate was oleate (19:1). A high concentration (22%) of polyunsaturate 22:6 was also present in the raw material, while the total unsaturates amounted to 60.8% (Table 2).

Proximate analysis carried out on vacuum packed, heat sterilised trenched sardines indicated a higher oil content present in the skin (5.96%) than the flesh (2.96%) (Table 2).

During the period of storage (75 days) of vacuum packed, heat sterilised fish, moisture content and pH showed little variation, while the oil content fluctuated with the mimimum value being 2.6% and maximum 4.3% in both lots of immediately iced and delayed iced fish (Table 4).

Peroxide values of immediately iced and delayed iced packs ranged from a minimum of 19.1 and 192 milli equivalents/kg oil to 103.2 and 95.9 milli equivalents/kg oil respectively at the end of the study (Table 5). During this period, no rancid odours or yellowing of liquid, oil or skin was observed in pouches processed from both immediately iced and delayed iced raw material (Table 4). There was a positive correlation between peroxide values of immediately and delayed iced, heat sterilised fish ($r = +0.8222$, $p \leq 0.05$ and $r = +0.9279$, $p \leq 0.05$) (Table 6).

A slight loss in flavour was detected in one batch of delayed iced fish towards the end of storage.

Vacuum packed, heat sterilised fish showed little change in fatty acid composition when compared with the raw fish. Total saturates were 41.7% with 29.2% being Palmitic acid. The notable feature was the increase of (16:1) and decrease of (18:2) in the pouched product. The difference may be due to the fact that for the raw fish only the flesh was analysed, while for the pouched product, the whole fish was analysed. It may also be due to variations between fish.

Net contents of the packs ranged from 170-191 g while the percentage drained mass was 75-77% (Table 7).

DISCUSSION

Dressed trenched sardines Amblygaster sirm, when packed in nylon/polyolefin pouches, sterilised at 121°C for 30 min and stored at 28±2°C gave a product with a shelf life of around 75 days. The major criteria in judging acceptability were visual and chemical parameters. Insignificant changes in moisture and pH during storage indicated the stability of the product during the period of study. Lee et al (1984) observed that fried mackerel meat paste vacuum packed in laminated plastic film, sterilised at 120°C for 20 min and stored at 25±3°C, kept in a good condition for 100 days, showing little change in pH, moisture and peroxide value. Similarly Lee et al (1983) had also observed that seasoned dried sea mussel (Mytilus edulis) vacuum packed in laminated plastic film and sterilised at 120°C for 25 min remained in a good condition for up to 70 days when stored at 35°C. Further, this product has also shown acceptability among the Sri Lankan consumer (Chinivasagam and Etoh, 1985).

The use of raw material from local landing sites obtained under traditional conditions seems to be suitable. Trenched sardines are a common and popular fish among the small shore seine catch. A delay of up to 5 h in icing was found to have no marked effect on the quality of trenched sardines (Chinivasagam and Vidanapathirana, 1985). In the present study the 5 h delay in icing gave a product of acceptable storage stability. Smith, Hardy and Young (1972) stated that at temperatures between 0°C amd ambient, 0^2 is obsilised by the fish in competition with microorganisms, enzymes and lipids.

The fatty acid profile of trenched sardine did not change during heat processing. Hale and Brown (1983) demonstrated that the fatty acid compositions of thread herring and Spanish sardine did not significantly alter during heat processing for canning. In the present study the satured fatty acids constituted 38.2% Palmitic (16:0) as the dominant fatty acid. This was comparable with the saturates of Malaysian (36-55%), New Zealand (36-42%) and Australian (30%) fish. The major monosaturate was oleate (18:1) as in Malaysian, Australian and New Zealand fish. The fatty acid 22:6 was high (22-24%) in trenched sardines as in Australian (max 37%) and New Zealand (18-27%) fish (Gibson, Kneebone and Kneebone, 1984; Hale, 1984 and Gibson, 1983). Evans, Fogarty and Sainsbury (1986) state that in the fish from the North-West shelf of Australia the 6 most frequently occurring fatty acids in order of their relative abundance were 22:6, 16:0, 18:1, 18:0, 20:4 and 16:1 which is similar to the pattern obtained for trenched sardines.

The fat content of trenched sardines varied from 3.9-6.5%. Ackman (1980) states that fat generallyu carries natural flavour components and provides and preserves other components generated during cooking, pickling or other processing. A gradual increase in peroxide values was observed both in packs processed from immediately iced and delayed iced raw materials, indicating oxidative changes taking place. However, during this period no signs of rancidity or yellowing was detected. Gruger (1967) states that rancidity is closely linked to the oxidation of unsaturated fatty acids present in the lipid fraction. A high percentage of unsaturates (58%) is present in this product.

The packs used in this study had low barrier properties for 0^2 and flavour vilatiles. This may account for the oxidation and slight loss of flavour in lot b of delayed iced fish. Thus the use of alternative packing material such as polyester/aluminium foil/polyolefin (oxygen transmission 0cc/m^2 day .atm at 27°C, 65% RM) would enable a product of longer shelf life (>75 days) but at a higher cost of production than the present packaging material.

Thus it could be stated that trenched sardines (and other small shore seine catches) could be preserved by retort pouching using either transparent or aluminium pouches, to provide an alternative processing method to freezing or drying. Also this product could be an alternative to canned food which is imported to Sri Lanka.

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Table 1

Moisture content, oil content and pH of immediately iced and 5 hour delayed iced raw material Amblygaster sirm

* Analysis	Immediately iced		5 hour delayed iced	
	a	b	a	b
Moisture	78.0	71.7	70.1	69.5
Oil	3.9	3.9	4.9	6.5
pH	5.9	6.0	6.0	6.0

* Duplicate analysis from a and b of immediately iced and delayed iced fish.

Table 2

Proximate composition of vacuum packed, heat sterlized, Amblygaster sirm

* Analysis	Ash (%)	Protein (%)	Oil (%)	Salt (%)	Moisture (%)
Flesh	2.4	26.6	2.96	2.0	67.2
Skin	1.1	25.7	5.97	0.9	69.0
Bones	20.6	15.8	-	0.8	56.6
Liquid	3.2	6.6	0.49	1.6	89.8

* Duplicate analysis from immediately iced fish

Table 3: Major fatty acids of unprocessed (flesh) and vacuum packed, heat sterilized *Amblygaster sirm*

Fatty acid	Percentage of total fatty acids	
	Raw flesh	Vacuum packed heat sterilized fish contents
14:0	6.0	4.6
16:0	22.4	29.2
18:0	9.8	7.9
14:1	1.4	1.0
16:1	1.4	10.8
18:1	12.4	7.9
20:1	4.4	0.7
22:1	2.2	2.7
18:2	9.2	0.7
18:4	-	-
20:5	7.6	5.4
22:5	-	-
22:6	22.2	24.6
Others	0.9	4.4
Total saturates	38.2	41.7
Total monounsaturates	21.8	23.1
Total polyunsaturates	39.0	35.1
Total unsaturates	60.8	58.2

Table 4 : Change in moisture, oil, pH and visual characteristics of vacuum packed, heat sterilized Amblygaster sirm processed using either immediately iced or 5 hour delayed iced raw material and stored at ambient temperature, ($28 \pm 2^{o}C$).

Days	* Moisture (%)		* Oil (%)		* pH (%)		Visual Characteristics	
	0 hr.	5 hr.	0 hr.	5 hr.	0 hr.	5 hr.	0 hr.	5 hr.
8	73.00	72.46	2.70	3.07	5.91	5.91	Unchanged	Unchanged
23	71.45	71.87	3.84	3.63	5.94	5.94	Unchanged	Unchanged
36	72.79	70.15	3.99	3.18	5.92	5.91	Unchanged	Unchanged
49	71.28	70.54	3.43	4.33	5.95	5.93	Unchanged	Unchanged
62	70.98	71.04	2.79	2.55	5.96	6.02	Unchanged	Unchanged
75	72.51	72.46	2.81	3.55	5.92	5.92	Unchanged	Slight loss of flavour

Unchanged - odour, flavour, appearance (yellowing) and texture remained unchanged.

* average of a & b from immediately and 5 hour delayed iced fish.

Table 5 : Change in peroxide value of vacuum packed, heat sterilized Amblygaster sirm processed using either immediately iced (0h) or 5 hour delayed iced (5h) raw material and stored at ambient temperature, (28 $\pm$ 2°C).

Days	Peroxide value (milli eq / kg oil)	
	0 hour	5 hour
8	19.10	19.27
23	38.82	39.87
36	55.96	58.77
49	56.37	44.99
62	48.93	56.62
75	103.24	95.88

Average of a & b of immediately iced and 5 hour delayed iced fish.

Table 6

Correlation coefficient between peroxide value of immediately iced fish and 5 hour delayed iced fish

	$a_o a_5$ peroxide value milli eq/kg oil	$b_o b_5$ peroxide value milli eq/kg oil
Correlation Coefficient	0.8222^x	0.9279^x

x Significant at 5% probability level.

Table 7

Percentage drained mass of cacuum packed, heat sterilized Amblygaster sirm

Pouch Code	1	2	3	4	5
Total Contents	185.3	179.3	191.7	190.4	170.7
Weight of pouch	4.6	4.5	4.5	4.5	4.5
Drained mass	138.6	133.2	144.8	140.5	129.0
Percentage drained mass	76.9	76.2	77.6	75.6	77.7

Using seive size 2.00 mm

FATTY ACID PROFILE

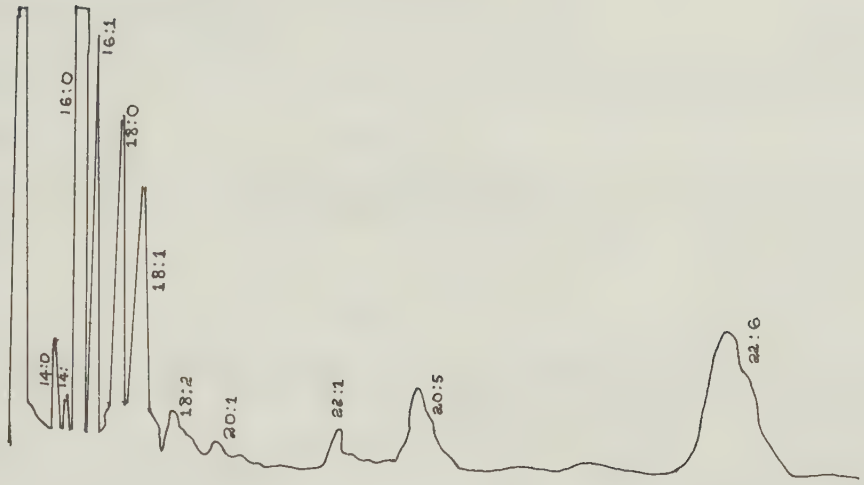

Fig. 1 RAW FISH, Amblygaster sirm

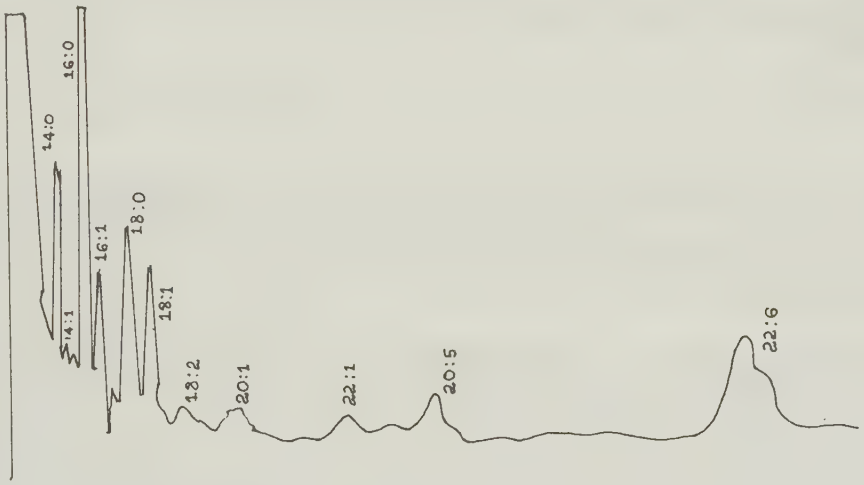

Fig. 2 VACUUM PACKED, HEAT STERILIZED FISH, Amblygaster sirm

STUDIES ON DEPURATION OF BIVALVES

by

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ABSTRACT

Indonesia, with vast coastal zones, has a great potential to develop mariculture including bivalve culture, especially blood cockles (Anadara sp.) ark (Anadara inflata) and mussel (Mytilus viridis).

However, the development of bivalve culture has been hindered by high bacterial load of the bivalve meat, especially when the surrounding waters are contaminated by domestic sewage.

For these reasons, studies on bivalve depuration have been carried out. The studies aimed to eliminate the pathogens and reduce the bacterial load of the meat.

A semi-commercial depuration unit was constructed for this purpose. The efficacy test was carried out using blood cockles, ark, and green mussels. The temperature of the surrounding water was maintained at 28°C, and the reduction of E. coli and V. parahaemolyticus counts were monitored.

Results showed that at 28°C the life span of ark, blood cockles and green mussel was 3, 2.5 and 2.5 days respectively, whereas the pumping rates were found to be 3.5 mL/h and 4.1 mL/h respectively.

Efficacy tests showed that after 3-day depuration, the E. coli count of the bivalves could be reduced from 386/g to 1/g for ark, from 333/g to 0/g for blood cockles and from >1,100/g to 0/g for green mussel. However, Vibrio parahaemolyticus counts remained constant for all the samples (more than 1,100/g).

INTRODUCTION

Indonesia, with vast coastal zones, has a tremendous potential to develop mariculture, including bivalve culture. Although there are so many species of bivalves which are suitable for cultivation, ark (Anadara granosa), cockle (Anadara inflata) and green mussel (Mytilus viridis) are among important species which show prospects for future development. To date most of the bivalves are harvested from natural beds, and only a small percentage are obtained from culture activities.

One of the main problems for the development of a bivalve mariculture programme is the high bacterial load and contamination with pathogens, mainly because of filter feeding. Previous workers indicated that bivalves are very susceptible to pathogens and viral contamination from the surrounding waters (Souness, 1979; Fujioka et al, 1981). There are also indications that bivalves tend to accumulate bacterial pathogens, viruses and other contaminants from the surrounding water into their meat (Souness, 1979). However, studies also indicated that bivalves are unable to clean themselves when put into clean water for at least 48 h. The Staphylococcus faecalis count could be reduced to 11 percent and E. coli count to 78 percent within 48 h (Thayib and Listiawati, 1979). Acording to Thayib et al (1977), more than 52 percent of Anadara sp. collected from Jakarta Bay were contaminated by Salmonella, 63 percent contained Shigella and 25 percent were contaminated by E. coli and 1 percent by Staphylococcus. Related studies also indicated that bivalves collected from Jakarta Bay were also subject to heavy metal contamination especially Hg, Cd, Pb, Bi, Zn and Cu. It is obvious therefore that there is an immedite need to find appropriate depuration techniques in order to minimise potential health hazard of bivalves for human consumption.

The present studies aimed to develop a semi-commercial scale bivalve depuration unit which could be used by local fishermen to reduce or eliminate pathogens from the bivalve meat.

MATERIALS AND METHODS

Ecological Conditions of Culture Areas

This study includes samples collected from different places known to be commonly used for bivalve culture. Samples collected include: surface water, sediment, fish and shellfish.

Depuration Apparatus

An experimental depuration unit was constructed, with a maximum loading capacity of 50 to 100 g of bivalves (Figure 1). The unit was equipped with a thermostat, water circulation pump and ultra violet water sterilizer.

Bivalves

Three species of bivalves were used in this experiment: ark (Anadara inflata) cockle (Anadara granosa) and mussel (Mytilus viridis), collected from Cilincing, Muara Angke and Ancol, which are adjacent to Jakarta Bay.

Seawater used in this study was furnished by the National Institute of Oceanology, which was pumped from Ancol waters prior to filtration.

Methods

Determination of pumping rates of bivalves

Pumping rate of bivalves were determined according to the method described by Souness (1979), i.e. by adding 10 ppm of neutral red into the surrounding water and measuring the uptake of the dye by the bilvalves at different temperatures. Due to unavailability of neutral red dye, brilliant blue at 10 ppm was instead used in this study. The stocking density was set at 4 bivalves/L or about 125 g of bivalves/L. Reduction of brilliant blue concentration in the surrounding seawater was then measured using spectrophotometer at 630 nm.

Efficacy tests on depuration unit

Prior to depuration studies, microbiological analysis of the bivalves was carried out, including total bacterial count, E. coli and Vibrio parahaemolyticus.

Since the E. coli count was found to be low, the surrounding water was intentionally inoculated with E. coli so that the E. coli count was between 10^8 to 10^9/mL. On the other hand, inoculation with V. parahaemolyticus was not carried out since the V. parahaemolyticus count of the mussels were quite high (11×10^2/g).

E. coli culture was isolated from mussel harvested from Ancol, using Endo Agar Medium (Town et al, 1970) and grown in 1% peptone water.

Total bacterial count was carried out in the initial and final stage of depuration studies, whereas E. coli and V. parahaemolyticus counts were carried out at 1, 12, 18, 36 and 72 h, using the most probable number technique (Diliello, 1980).

RESULTS AND DISCUSSION

Ecological Conditions of Culture Areas

Results of ecological studies of the culture areas are given in the following tables.

Table 1

Heavy metal contents of surface waters of Jakarta Bay
(ppm)

Heavy metals	Concentration (ppm)	Standard EPA	Standard FWPCA	Standard NASE
Hg	0.25 - 73.0	0.1	0.03	0.01
Cd	0.5 - 450.0	10.0	-	0.2
Pb	5.0 - 500.0	10.0	0.03	10.0
Bi	12.5 - 594.0	7.0	-	2.0
Zn	14.2 - 749.9	10.0	-	20.0
Cu	0.5 - 80.0	-	30	-

Note: EPA - Environmental Protection Agency
FWPCA - Federal Water Polution Control and Administration
NASE - National Academy of Science and Engineering

Table 2

Positive results of pathogenic bacteria from samples collected from the Jakarta Bay and vicinity

Bacteria isolated	Samples					
	Sediment		Fish		Shellfish	
	Total	% pos.	Total	% pos.	Total	% pos.
Clostridium sp	600	6.3	62	3.2	49	0
V. parahaemolyticus	40	15	196	9.7	254	13.4
Salmonella sp.	15	6.7	68	4.4	300	52.3
Shigella sp.	-	-	68	5.9	300	6.3

Table 3

Bacterial counts of green mussels harvested from Jakarta Bay and vicinity

Location	Total Plate Count	Coliforms	E. coli
A			
- green mussels	5.8×10^5/g	920/g	8.1/g
- water	9.7×10^3/mL	240/mL	1.5/mL
B			
- green mussels	3.7×10^4/g	84/g	3.9/g
- water	1.5×10^3/mL	0/mL	0/mL
C			
- green mussels	8.5×10^7/g	1800/g	250/g

Note: A: samples collected 100m away from Ancol Beach, Jakarta
B: samples collected 1 mile away from the beach
C: samples collected from fish market which had been harvested by local fishermen from the Bay

Further studies of the ecological conditions for bivalve culture in Cilamaya coastal waters indicated that mercury was not detected in seawater, but the mercury level in the cockles was found to be 0.264 ppm. Blood cockles harvested from this area have significantly high bacterial load and Staphylococcus counts of 80×10^6 and 10^7 per gram respectively. Moreover, the coliform counts were also found to be high although E. coli were absent.

Studies on the quality of cockles harvested from Serang and Subang waters (W. Java) have also been carried out. The results showed that the maximum mercury content of the cockles was 0.264 ppm especially in "kerang tahu". Bottom culture of cockles in Subang waters resulted in lower quality raw materials with high bacterial load, high mercury and TVB content. On the other hand, cultured cockles using raft system in Serang waters produced relatively good quality cockles. Faecal coliforms were not detected in any samples.

Evaluation of mercury content of "market" cockles from retailers at Muara Karang, Jakarta indicated that the mercury level of all samples were still below the permissible limit of 0.5 ppm. Green mussels collected from this market showed a mercury content of 0.451 ppm. Furthermore, these green mussels have the highest value of pH, TVB and moisture content, i.e. 6.3, 13.9 - 15.9 mg n% and 81.5% respectively. Microbiological analysis indicated that the highest bacterial count was found in blood cockles, although faecal coliforms were found to be absent.

Pumping Rate of Bivalves

The results showed that the pumping rate of the bivalves increased with increasing water temperature. It was also noticed that the pumping rate of cockles (Anadara granosa) was the highest (5.4 mL/h), followed by green mussel (M. viridis) (4.1 mL/h) and ark (A. inflata) (3.5 mL/h).

Table 4

Pumping rate of mussels at different temperatures

Species	Pumping rate (mL/h) 26°C	27°C	28°C	29°C	30°C
Cockle	1.1	1.1	1.8	2.1	2.1
Ark	2.4	2.7	2.7	3.0	3.4
Green mussel	1.4	1.8	2.1	2.1	3.4

At 26 and 27°C ark could survive for 3.5 days, whereas green mussels and cockles both survived for 3 days. At 28°C on the other hand, ark, cockle and green mussel could survive for 3, 2.5 and 2.5 days respectively. The survival rate of the bivalves at 29°C was 2.5, 2.5 and 2.0 days for ark, cockle and mussel respectively, whereas at 30°C the rate of survival was 2, 1.5 and 1.5 days respectively.

These results indicated that the optimum depuration temperature was around 28°C, since at this temperature the survival rate was still more than 2 days, and the pumping rate was relatively high.

Depuration Studies using the Laboratory Scale Depuration Unit

Green mussels collected from Ancol Bay, Jakarta, were used for experimental studies. The mussels were sprayed with clean seawater to remove mud, sand and other debris and put into plastic trays in the depuration unit. The depuration unit was then filled with seawater which was pre-inoculated with pure culture of E. coli and the water was allowed to circulate for 1 h without u.v. light. The depuration process was then carried out for 48 h.

Results showed that the laboratory scale depuration unit equipped with a 90 watt u.v. light was quite effective in reducing the total bacterial load, coliforms and E. coli contents of green mussels. After 48 h, the bacterial load, coliforms and E. coli count of the mussels decreased from 10^7 to 10^5 cells/g, 82 to 8.9 cells/g and from 45 to 1.79 cells/g respectively. The optimum allowable density of green mussels under this condition was 3 green mussels/L of water.

Table 5

Reduction of bacterial load of the water during depuration process without u.v. light

Time (hours)	Bacterial count/mL			
	TCP	Coliform	E. coli	Percentage of E. coli reduction
0	1.8×10^6	1800	49	0
12	4.3×10^5	350	24	51.0
24	2.4×10^5	250	20	59.2
48	3.0×10^4	72	12	75.5

Table 6

Effect of depuration using 30 watt u.v. light on bacterial load of the water (count/mL)

Time (hours)	TPC	Coliform	*E. coli*	Percentage of *E. coli* reduction
0	1.2×10^5	920	49	0
24	7.6×10^4	23	7.8	84
48	7.0×10^4	0	0	100
72	4.2×10^4	0	0	100

Table 7

Effect of depuration using 60 watt u.v. light on the bacterial load of the water (count/mL)

Time (hours)	TPC	Coliform	*E. coli*	Percentage of *E. coli* reduction
0	1.3×10^6	1800	62	0
6	7.6×10^5	240	26	56
18	2.5×10^4	48	11	82
24	4.2×10^3	0	0	100
48	4.9×10^3	0	0	100

Table 8

Effect of depuration using 90 watt u.v. light on the bacterial load of the water (count/mL)

Time (hours)	TPC	Coliform	*E. coli*	Percentage of *E. coli* reduction
0	3.4×10^5	1800	70	0
6	1.6×10^5	95	11	84
12	3.5×10^4	46	7.6	89
18	4.0×10^3	0	0	100
24	2.9×10^3	0	0	100
48	4.8×10^2	0	0	100

Effectiveness of the Commercial Scale Depuration Unit

As shown in the following tables, soon after inoculation with E. coli, the E. coli count in the bivalves was gradually increased. Even after 2 hours of depuration the E. coli count in the ark and cockle meat had increased. However, the E. coli count quickly declined after 12 h of depuration.

Table 9

E. coli counts of bivalves during depuration process

Sample	E. coli count/g of meat					
	Before inoculation	After inoculation	12 h	36 h	60 h	72h
Ark	125.3	386.7	526.7	14.8	4.2	1.2
Cockle	191.0	333.3	264.3	13.1	3.0	0.0
Mussel	15.0	>1100	>1100	50.3	6.7	0.0

Note: Figures are average of troplicates.

As shown in the following table, V. parahaemolyticus counts of ark, cockle and bivalve remain constant during depuration, i.e. more than 1,100/g. It appears that the depuration unit was not effective in reducing V. parahaemolyticus from the bivalves.

Table 10

V. parahaemolyticus counts of bivalves during depuration process

Sample	Initial	12 h	36 h	60 h	72 h
Ark			All samples > 1100		
Cockle			All samples > 1100		
Mussel			All samples > 1100		

Note: Figures are average of triplicates.

As shown in Table 11, the total bacterial counts of the bivalves meat were also significantly reduced.

Table 11

Total bacterial count of bivalve meat/g during depuration process

Species	Time	Samples			
		Sample 1	Sample 2	Sample 3	Sample 4
Ark	0 h	3.4×10^5	3.4×10^5	3.3×10^5	3.3×10^5
	72 h	1.2×10^5	1.5×10^5	9.3×10^4	1.2×10^5
Cockle	0 h	1.2×10^5	9.4×10^4	9.4×10^4	1.0×10^5
	72 h	4.8×10^4	7.6×10^4	7.9×10^4	6.7×10^4
Mussel	0 h	3.8×10^4	6.6×10^4	4.7×10^4	5.0×10^4
	72 h	3.5×10^4	4.9×10^4	4.4×10^4	4.2×10^4

CONCLUSIONS

Bivalves tend to accumulate microbes and other contaminants from the surrounding water, mainly due to their filter feeding process.

Depuration using u.v. light could be effectively used to eliminate total bacterial load and E. coli content of the bivalve meat, but ineffective against V. parahaemolyticus.

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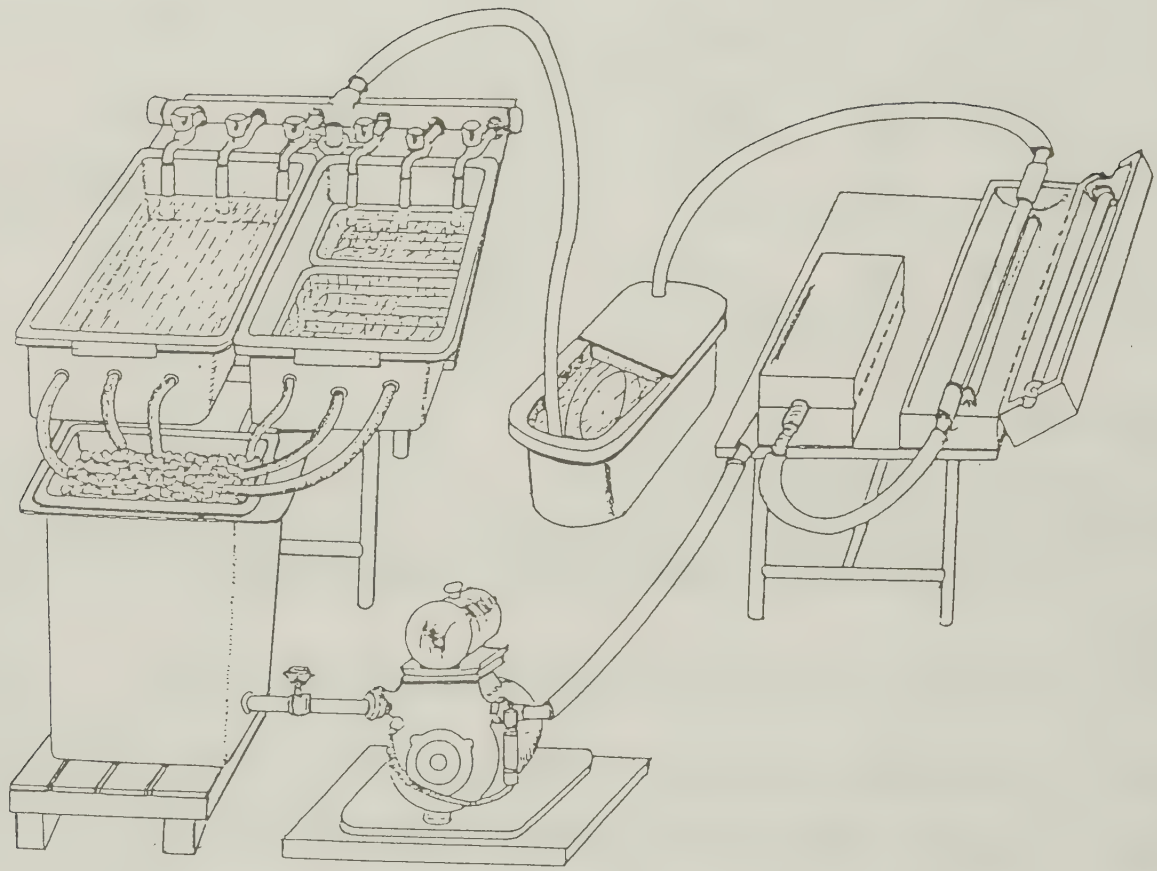

Figure 1 Laboratory scale of depuration unit

OYSTER DEPURATION - A CASE STUDY

by

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ABSTRACT

By virtue of their filter feeding system shellfish accumulate human pathogenic bacteria and viruses when grown in sewage polluted waterways. On transfer to disinfected water, shellfish will eliminate the previously accumulate microorganisms with their faeces as part of their feeding activity and become microbially cleansed. This process is termed depuration.

This describes the process by which an appropriate depuration system is developed from laboratory experiments through field trials to full-scale commercial application. Sydney rock oysters (Crassostrea commercialis) caused a significant food poisoning incident in 1978 involving 2,000 people. A depuration system developed to overcome the water pollution problems is presented as a case study; the principles developed for oysters can be applied to other shellfish.

1. INTRODUCTION

Shellfish have a well documented history of causing food poisoning and transmitting human disease. These conditions include typhoid, paratyphoid, cholera, dysentery and gastroenteritis (Fleet, 1978) .

The public health risk associated with shellfish is attributable to certain aspects of shellfish physiology, production and consumption, which may be summarized as follows:

1. Shellfish are filter feeders, a process which concentrates microorganisms which are found in the marine environment, in their gut.

2. Marketing procedures often mean shellfish may spend long periods out of water in high ambient conditions which encourage the multiplication of contaminating microorganisms.

3. Various culinary habits often mean that shellfish are either consumed raw or with minimal cooking.

Several methods have been adopted worldwide to eliminate microorganisms from shellfish and these include:

1. Certification of growing areas so that shellfish are only harvested from pollution free waterways.

2. Relaying of shellfish, which is the transportation of shellfish which have been grown in polluted waterways to areas certified free of pollution for a period to permit microbiological cleansing.

3. Depuration which is a process by which oysters grown in polluted waters are placed in tanks of sterile water for a period to allow cleansing. Due to the security and flexibility offered by depuration this method of shellfish cleansing is the preferred method worldwide.

2. CASE STUDY

2.1 Background

During June and July 1978, oysters were definitely implicated in a major outbreak of gastroenteritis involving more than 2,000 people throughout Australia. The oyster involved was the Sydney rock oyster (Crassostrea commercialis) which is cultivated in the estuaries of New South Wales. Viral contamination of oysters through sewage pollution of their feeding waterways was suspected of being the causative factor. This outbreak highlighted the need for the development of

oyster depuration technology suited to Australian conditions. Up to this time oysters grown in Australian waters were considered safe to consume and were in fact exported worldwide. The oyster industry was threatened with financial ruin as a result of the adverse publicity arising from this incident. The oyster industry united to provide funds to the University of New South Wales in order to develop a suitable depuration system.

Various forms of depuration have been practiced in Europe and America for many years. The decision was made when planning the research program not to simply import the necessary technology but to develop a depuration system suited to Australian environmental conditions and the local oyster.

Shellfish cleanse themselves during depuration as a function of their feeding, digestion and excretion process. Microorganisms from the gut are encapsulated in the mucous matrix of the faeces and excreted as part of the normal process of digestion. Any factor which has a negative impact on this process would result in inefficient depuration and therefore the production of an unsafe product.

The limiting factors that would affect depuration were identified as follows:

1. Shellfish condition which can be affected by excessive storage time out of water, rough handling and damage to the shellfish prior to depuration, can result in stress or death. Moribund or dead oysters will not feed and so cannot be cleansed.

2. Environmental factors have a significant affect on shellfish feeding rate and hence cleansing rate. These factors are:

- Water temperature; lowering temperature below ambient will reduce cleansing rate, while increasing temperature can encourage spawning and a rapid increase in the multiplication rate of microorganisms.
- Fluctuations in water salinity although not critical, may temporarily affect cleansing rate.
- Also a satisfactory level of dissolved oxygen is necessary to maintain acceptable cleansing rates. Oxygen levels depend upon shellfish/water ratio, water temperature and rate of flow of water through the system.
- water turbidity can be an important factor as excessive turbidity reduces water sterilising effectiveness and may lead to reduced shellfish activity.

3. System design is the last limiting factor. Tanks, pumps, pipelines and holding tanks should be constructed of materials and in a way that does not stress shellfish and encourages maximum activity and therefore cleansing rate.

2.2 Research Description and Results

The research program was divided into three components (Souness and Fleet, 1979) . Laboratory scale studies to quantify those environmental factors mentioned previously. Followed by field trials to test findings of the first component and to finalise the system design. Lastly two commercial scale plants were constructed which demonstrated the effectiveness of the final design and to test the economics of the system.

In designing a laboratory depuration system a decision had to be made on the mode of water sterilisation. There are several treatments available:

- chlorination
- ultra violet radiation
- ozonation
- membrane filtration
- iodofors

Each treatment has its limitations. After considering the results presented in the literature and our proposal to design a system that would require minimal maintenance and operator training, UV radiation was chosen as the most appropriate treatment.

UV near 254 nanometres wavelength has a potent, destructive effect upon bacteria and viruses. It is an effective method of continuously sterilising large volumes of water. Water turbidity can reduce the antimicrobial action of UV but this can be overcome by the inclusion of filters.

The laboratory system consisted of glass tanks (67x30x30 cm), a centrifugal water pump, a UV water steriliser, an aeration device and system for maintaining water temperature. Water was recirculated by the pump from the tank, through the UV steriliser and then a spiral of glass tube immersed in a constant temperature waterbath and returned to the tank. Dissolved oxygen was monitored regularly and salinity, once set, remained constant. Pumping rate and hence recirculation could be set and maintained at a constant rate.

Before the system was operated with oysters the effectiveness of the UV steriliser was assessed. This was done by contaminating sea water with a range of bacteria, taking a sample, passing the water once through the UV unit and taking another water sample. Bacterial counts (Table 1) on the samples demonstrated the unit was capable of destroying greater than 99% of the challenging bacteria on a single pass.

The optimal oyster pumping rate was assessed by a dye uptake technique (Cole & Hepper, 1954) for a range of water temperatures. When oysters are immersed in a solution of neutral red the dye is absorbed into the oyster flesh at a rate proportional to oyster pumping rate. Pumping rate can be assessed by measuring the amount of dye remaining in the solution spectrophotometrically. The pumping activity for the Sydney rock oyster is presented in Figure 1, which shows that little activity occurs below 15°C while optimal activity occurs at 25°C.

The purpose of the laboratory oyster depuration tests was to determine correct operating parameters for Crassostrea commercialis. Oysters were harvested from polluted waterways and brought directly to the laboratory for microbial analysis. They were then placed in the tank and water recirculated through the system described previously. The depuration water was taken from the same waterway as the oysters were taken to ensure minimal stress. Salinity was measured at 3.1 g/L and oxygen was maintained at greater than 70% saturation.

Trials were conducted using water at 19°C and 28°C. At the higher temperatures the rate of bacterial elimination decreased and the bacterial counts of the tank water increased despite the UV steriliser operating efficiently. Several explanations include:

- increased rate of bacterial multiplication in tank water.
- selection of UV resistant bacterial species within the depuration water.
- at high temperature the mucous matrix of oyster faeces break down releasing entrapped microorganisms back into the depuration water.

The principle method of controlling bacterial build-up is by adjusting the recirculation rate thus maximising the exposure of the water to the UV light. The limiting factor controlling recirculation rate is the sterilising capacity of the UV sterilising unit. During laboratory trials recirculation rate was varied from 1 to 4 cycles/h. One cycle is the volume of water in the depuration system. These tests at 20 and 25°C showed that bacterial build-up could only be controlled at 20°C and 3 cycles/h.

The final factor that requires attention is the ratio of oysters to water volume in the depuration tank. Obviously, to optimise the depuration capacity of a system, it is desirable to have as many oysters in a tank as possible without affecting depuration rate. Tests were done at 2 and 4 oysters/L and no significant difference in depuration rate was noted. Densities in excess of 4 oysters/L required stacking of oysters on top of each other which inhibits oyster feeding and hence depuration.

The pilot scale unit consisted of a rectangular fibreglass tank capable of holding approximately 1,200 oysters which represents a commercial bag of oysters. The oysters were held in the tank on three plastic meshed trays which each held 400 oysters. These trays could be conveniently handled by one man, while the mesh allowed the unimpeded flow of sterilised water and for faeces to fall away from the oysters to the base of the tank.

Using a depuration temperature of 20°C, 3 tank water cycles/h and an oyster concentration of 3 oysters/L, 1250 oysters were successfully depurated using the pilot scale system. The results presented in Table 2 show that on this scale, oysters cleansed themselves of indicator organisms and total plate count was reduced 10 fold within 48 h. As found with laboratory trials, circulation rates less than 3 cycles/h and water temperatures in excess of 25°C gave rise to bacterial accumulation in tank water.

The basic information necessary for the development of a commercial depuration plant applicable to the Sydney rock oyster was finalised through these trials. This information is given in Table 3.

Following-the successful testing of the pilot scale unit the input of the local oyster industry was canvassed in relation to plant design. Basic requirements included:

- capacity between 5,000 and 20,000 oysters per cleansing operation
- low capital cost with economy of space
- low running and maintenance costs
- simplicity of operation and ease of handling

It was considered that an amplification of the pilot scale unit would meet these requirements. A depuration tank was designed to ensure ease of operation and efficient depuration. The tank contained 3 sections (Figure 2):

- the inlet section consisting of a baffle and overflow weir
- main body in which oysters are located
- water outlet section consisting of an overflow weir and drainage hole.

The inlet section was designed to produce a constant and uniform flow of water through each tank, so that the water volume in the tank was exchanged every 15 to 20 minutes. The flowrate into each tank is controlled by the area of holes in the baffle and the pressure drop which is equal to the difference in height between the overflow weirs in the inlet and outlet sections. Since these parameters are fixed by the design, the flowrate through the tank is independent of the amount of water entering the reservoir. Another important benefit of this tank design is its contribution to uniform water flow through the tanks, thus ensuring equal exposure of all oysters in a tank to sterilised water. Therefore oysters are depurated at an approximately similar rate.

This design was possible due to the use of vertically stacked tanks, which relies on gravity to motivate the water down through the tanks. Stacking has several other benefits which include:

- the economic use of space
- the free falling of water from tank to tank both oxygenates the water and cools it by several degrees.

Two commercial depuration plants were designed and built using the design criteria developed from the laboratory and field tests. The first plant consisted of 12 tanks, stacked in 2 groups of 6 and holding 15,000 oysters while the second, larger plant consisted of 2 stacks of 18 tanks with a capacity of 45,000 oysters.

Both plants were assessed for depuration efficiency. Samples of oysters and water were randomly taken from different tanks, at different times and examined for total plate count and *E. coli*. The results presented in Table 4 show that heavily polluted oysters were cleansed to acceptable standards within 48 h.

The criterion for microbial safety should be conformation to current microbiological standards, which for oysters in Australia are (NH & MRC, 1976) :

- *E. coli* 2.3 cells/g
- Total count 10^5 cells/g

3. CONCLUSIONS

The responsibility for meeting these standards rests with the processor and it is the function of public health authorities to enforce the standards.

The following critical points arise in depuration of shellfish (Souness et al., 1979) and should be given consideration when formulating a code of practice:

- gentle handling of shellfish to ensure active feeding during depuration
- maintenance of at least 3 exchanges/h of water through depuration tanks and sterilisers
- heating and cooling facilities to maintain operating temperatures
- a depuration cycle not less than 48 h
- sufficient water sterilising capacity to cope with peak microbial loads
- regular inspection of sterilising equipment
- cleaning and sanitation of tanks, pipes, pumps and sterilisers on a regular basis.

A final bonus derived from the depuration of shellfish is a significant increase in shellfish viability out of water. The results presented in Table 5 demonstrate the impact of depuration on storage life. Oysters depurated for 48 h produced a 5% mortality rate after 3 weeks storage while the same oysters that were not depurated produced a 50% mortality rate after a similar storage period.

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Table 1

UV light disinfection of sea water

Water sample	Bacterial Counts (cells/ml) Before UV exposure	After UV exposure	% reduction
Before addition of oysters	800	0	100
After addition of oysters	$3x10^4$	20	99.93
After 48 h depuration	$2x10^4$	600	97

Table 2

Pilot scale depuration results

Depuration Time (h)	Cells/g oyster Total count	Coliforms
0	$12x10^3$	46
101	$4x10^3$	4.3
30	3x10	2.3
48	$2x10^3$	2.3

Table 3

Parameters necessary for depuration of the Sydney rock oyster

Parameter	Value
Water temperature	18 - 22°C
Water flowrate	3 tank volumes/h
Oyster density	4 oysters/L
	599 oysters/M^2
Salinity	100±20% of value at harvest area
Dissolved oxygen	50 - 100% of saturation

Table 4

Bacterial counts for oysters and water sample from a commercial depuration plant

	Bacterial counts (cells/g or mL)		
	Oysters		Water
Depuration time (h)	Total count	*E. Coli*	Total count
0	$15x10^4$	4.3	$13x10^4$
24	$1.2x10^4$	0.9	$1.8x10^4$
48	1.0x104	0	$1.1x10^4$

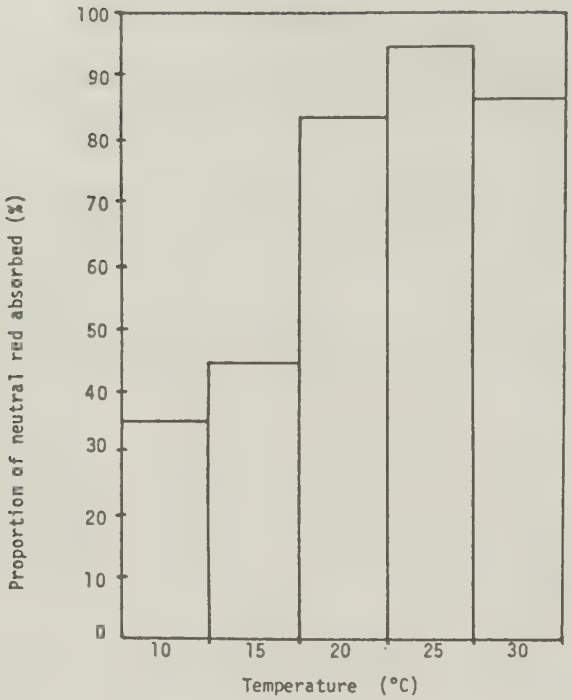

Figure 1. The effect of temperature upon the pumping rate of *Crassostrea commercialis*, expressed as dye uptake after 4 h.

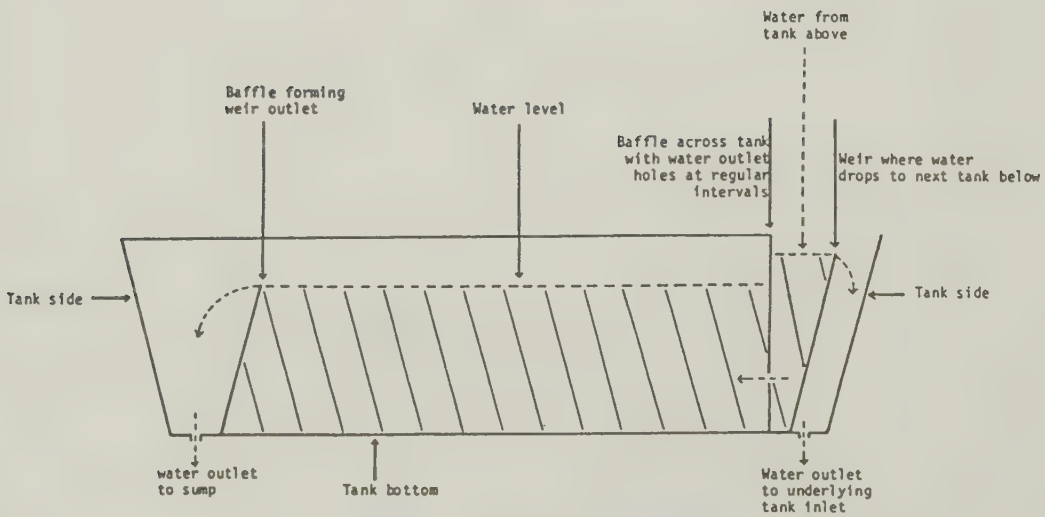
Water from tank above
Baffle forming weir outlet
Water level
Baffle across tank with water outlet holes at regular intervals
Weir where water drops to next tank below
Tank side
Tank side
water outlet to sump
Tank bottom
Water outlet to underlying tank inlet

Figure 2. Cross-section showing details of Phillips' tank.

POTENTIAL OF SURIMI PROCESSING IN THAILAND

by

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ABSTRACT

The suitability of a number of species available in Thailand for surimi production was investigated. The quality and gel strength of surimi was influenced by species and quality as well as by the technology used, the leaching techniques, temperature control and duration of mixing. Big eye snapper (Priacanthus) was the best species for surimi.

1. INTRODUCTION

Surimi based products are real, not imitation, foods high in protein and rich in nutrients, with a pleasing taste, having less fish odour and lower cholesterol (19.7 mg/100 g) compared with 151.1 mg/100 g in shrimp, 41.7 mg/100 g in crab and 29.7 mg/100 g in fish (Alaska pollock) (Beacham et al., 1984). Many types of demersal fish can be used for surimi; for example we have tested threadfin bream (Nemipterus sp.), Monocle bream (Scolopsis sp.), Goat fish (Mullidae sp.), big eye snapper (Priacanthus sp.), jewfish (Promicrops sp.), pony fish (Leognathus sp.) and flathead fish (Cynoglossus sp.).

Since 1986, more than 10 raw surimi processing plants have been established in Thailand. About 10,000 t of surimi product is exported annually, mostly to Japan and Singapore and crab leg analog has been produced from Thai surimi. The gel strength is 920 g cm^{-2} (using Fudo Rheometer with 3 mm head plunger). Many surimi based fast food products have been produced by hand for local consumer tests. The surimi products were accepted because they are familiar, cheaper than imported crab, tasty, convenient and buyers are also influenced by fashion trends. Fortunately for Thailand the alaska pollock harvest has been limited; so, not only the Japanese market is open for Thai surimi products, but also Singapore, Hong Kong, EEC and the USA. The USA is expected to reach $3 billion in consumption in 1990. Surimi products are a viable opportunity for the Thai private sector because of the fast growing market. Not only are the fish cheap in Thailand but the cost of labour is competitive. The main problem is to attain end-product quality that meets international standards.

There are several species of Thai fish which can be used to produce good quality of surimi, which give good gel strength and taste. Fifteen years ago only surimi type fish balls were popular even though the quality was below standard. Only 2 to 3 surimi plants produced 2,000-3,000 t of raw surimi annually, mostly for Japan. Threadfin bream, big eye snapper, monocle bream, croaker, goat fish totalling more than 100,000 t are now harvested annually. Since 1985, surimi production techniques have been transferred to the private sector and now 12-13 surimi plants have been set up, nine of these have received BOI privileges. The big surimi markets are Japan, USA, Australia, Singapore and Hong Kong. The reduction in the Alaska pollock catch by Japan, has opened new markets for Thai surimi.

Not only does fish quality and species type affect the gel forming characteristics of the final product, but also the technology used, leaching techniques, temperature control, mixing duration, as well as the mix of fish species used plus other ingredients. All of these variables have been the subject of laboratory studies.

2. MATERIALS AND METHODS

Fresh threadfin bream (Nemipterus sp.), big eye snapper (Priacanthus sp.), croaker (Pseudociaena sp.), Goat fish (Mullidae sp.), flathead (Cynoglossus sp.), flat fish (Leognathus sp.), pony fish (Leognathus sp.), lizard fish (Saurida sp.), sardine (Sardinella sp.) and also the frozen threadfin bream and lizard fish were tested for differences in the quality of surimi produced from these fish. The surimi was tested for gel-strength, whiteness, colour, odour and flavour.

2.1 Equipment

Deboner machine
Washing tank
Screw press dehydrator
Strainer
Mixer (kneading machine or silent cutter)
Water baths with temperature control
Freezer

2.2 Chemical and Microbiological Analysis

TVB by micro-diffusion
Fudo rheometer
pH meter
Petri dish for standard plate count and media, reagents
Water baths
Incubator
Microscope

2.3 Procedures

The fish was processed as in chart I and kept in 5-10 kg blocks in plastic wrap, frozen by contact freezer then stored at -25°C. The cooked surimi of 2.5 cm diameter was tested for gel strength and chemical and microbiological analysis was performed. Samples drawn from fish, after deboning, washing, dehydration, mixing of ingredients, freezing and after cooking were analysed for total volatile base (TVB), pH, K-value, and microbiological characteristics were determined at the same time as chemical analysis.

3. RESULTS AND DISCUSSION

The results showed that the fish and surimi product (Table 4) were high in protein content, low in fat (except sardine) and TVB indicated that the quality of fish was acceptable (10.52-16.37 mgN/100 g). Sardine was found to produce a darker coloured surimi and there is a need to adjust the pH by soaking with sodium-bicarbonate.

Big eye snapper surimi is of top grade for surimi production in Thailand, it's pH is 6.77 giving a 832 g cm^{-2} gel strength (with 5 mm head plunger) fold test was AA.

It was found that total viable bacterial count (TVB/g) was high (Table 7) after each step in the processing chain but our current method of surimi processing is not the most modern. We would expect much lower bacterial counts after installing our new surimi pilot plant.

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Chart 1. Raw Surimi Production Chain

Threadfin bream and others

Washed - cleaned

Gutted, deheaded and properly washed

Deboned

Fleshed : washed

(clean, cold water + 0.2% salt)

(Wash water : fish) = 5-7 : 1

rinse and dehydrate
each replication

2-3 replications per test

Dehydration with

screw press dehydrator

Strainer

Mixed with cryoprotectants

(sugar, sorbital and sodium polyphosphate)

Vacuum pack in plastic

Keep in $-20^{\circ}C$ cold room

Chart 2. Crab Leg Production

Raw Surimi

Silent Cutter

Mixer

Ingredients

- 10% starch
- 5% egg white
- 0.5% sodim monoglutamate
- 30% - 40% water (by weight)
- 0.2% flavour
- 0.3% salt
- 3%-5% sugar
- 100% Surimi

Sheet pump

Steam, heat

Fabrication

Rope forming

Colouring

Cutting

Packing

Steaming

Drying

Repacking

Freezing

Table 1

Thai fishery products and frozen fish exports, 1981-1986

	1981	1982	1983	1984	1985	1986
Quantity (tons)	56,856	55,217	53,410	75,254	96,444	118,907
(%) of Total	17.8	16.8	15.5	18.3	20.8	19.7
Value (million baht)	676	689	685	1,017	1,377	2,174
(%) of Total	8.4	6.1	5.4	6.7	7.5	8.1
Total fishery exports (tons)	320,325	316,597	344,899	411,722	464,792	602,490
Total value (million baht)	9,102	11,231	12,677	15,080	18,457	26,825

Table 2

Pacific pollock used for Surimi (x 1,000 tons)

	1979	1980	1981	1982	1983	1984
Japan	1,551	1,551	1,595	1,570	1.141	1,625
Korea	297	285	279	262	367	399
USSR	2,049	2,112	2,138	2,498	2,447	3,450
TOTAL	3,994	4,021	4,172	4,475	5,047	3,273

Source: Globefish

Table 3

Japanese crab-analogue export (tons)

	1981	1982	1983	1984	1985
U.S.A.	2,228	6,749	13,023	26,756	22,712
Australia	1,730	1,750	1,500	1,681	1,192
U.K.	-	348	2,130	1,602	1,394
Netherlands	4	117	241	269	335
TOTAL	4,044	9,330	18,828	32,462	36,692

Source: MMFS U.S.A.

Table 4

Proximate composition of 9 species of fish

			Moist	Prot.	fat.	TVB	pH
	1.	Threadfin beam (Nemiterus sp.)	81.02	18.14	0.32	14.66	6.86
	2.	Big eye snapper (Priacanthus sp.)	78.58	17.87	0.72	11.42	6.73
	3.	Croaker (Pseudociaena sp.)	80.29	18.11	0.62	10.52	6.50
	4.	Goat fish (Mullidae sp.)	79.44	11.52	0.44	14.66	6.64
	5.	Flat head (Cynoglossus sp.)	81.48	18.91	0.23	18.32	6.45
	6.	Flat fish (Liognathus sp.)	80.75	14.91	0.76	13.11	6.40
	7.	Pony fish (Leiognathus sp.)	81.98	17.00	0.86	11.93	6.34
1/	8.	Lizard fish (Saurida sp.)	82.16	15.22	4.46	13.89	6.41
2/	9.	Sardine (Sardinella sp.)	78.64	13.42	11.45	10.64	6.25

1/ = need improved techniques to get good gel strength
2/ = need sodium bicarbonate to adjust pH up to 6.6 - 7.0

Table 5

pH and gel strength of good quality of surimi from threadfin and big eye snapper for crab analogue

	pH	Gell strength gm/cm	Fold test (%)	Grading for freshness
Threadfin bream	6.93	491	AA	62
	6.71	381	A	62
	6.89	666	AA	85
	6.88	572	AA	82
Big eye snapper	6.71	703	AA	85
	6.77	832	AA	82

Table 6

Nutritional composition of the surimi analogue compared to Alaska king crab and surimi analogue

	Alaska pollock	Surimi pollock	Alaska king crab	Surimi crab analogue
Protein (%)	14.45	15.18	15.37	21.02
Calcium (mg/100 gm)	4.90	8.70	73.10	13.40
Potassium (mg/100 gm)	311.00	78.00	204.00	90.00
Niacin (mg/100 gm)	1.36	0.22	1.10	0.18
Cholesterol (mg/100 gm)	47.50	29.70	64.70	19.70

Source: National Food Processors Association
1401 New York Avenue, N.W.
Washington, C.C. 20005

Table 7

Microorganisms contamination of fish, water, surimi and crab-stick at each step of the process

	TVC/gm		Gram - negative rod CFU/gm	Entero bacteria-aceae CFU/gm	Pseudo-nas CFU/gm	E.coli CFU/gm	Strept. faecalis CFU/gm
	20°C	37°C					
Pla (fish)	5.95×10^4	1.08×10^5	-	1.0×10^2	-	0	-
First wash water	1.27×10^6	9.86×10^5	7.35×10^3	4.0×10^3	4.0×10^0	1.0×10^1	2.85×10^2
Third wash water	4.35×10^4	2.71×10^5	1.20×10^3	3.9×10^3	1.0×10^0	5.5×10^0	3.4×10^1
Fifth wash water	2.56×10^5	1.41×10^5	2.25×10^3	2.0×10^2	0	5.0×10^0	5.5×10^0
Surimi	2.35×10^6	1.63×10^6	-	8.4×10^3	-	6.5×10^1	-
Crab-stick (by hand)	-	8.5×10^6	-	7.3×10^5	-	5.2×10^0	-
Crab-stick (steam)	-	3.8×10^3	-	2.3×10^2	-	-	-
Frozen crab stick (after steaming)	-	1.0×10^0	-	-	-	-	-
Crab sheet frozen in bags	-	1.0×10^1	-	-	-	-	-

Staph. aureus was only found 1.15×10^3 CFU/gm in first wash water of minced fish but not in third and fifth wash water.

AN IMPROVEMENT OF HANDLING TECHNIQUE AS AN EFFORT TO INCREASE THE MARKETING OF CHILLED TUNA IN INDONESIA

by

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ABSTRACT

The Indonesian fishery sector has proved an important source of foreign exchange. Within the fourth Five Year Development Program, Indonesian fishery exports in 1987 increased in both value and volume. During the period January - September 1987 exports of fishery commodities was 97,554 t with a value of US$430 million, an increase of about 35% in volume and 31.5% in value against the same period in 1986.

Among the export commodities, chilled tuna has become a prominent fishery product following frozen shrimp and frozen tuna. There is a great demand for chilled tuna on the Japanese market, which can absorb as much as 200 t of fish per day. Being in high demand, chilled tuna attracts a relatively high price (current price is US$3300-4000/t) compared to frozen tuna which is only US$900-1000/t).

Chilled tuna is exported mainly to Japan and will usually be processed into sashimi and since sashimi is consumed raw, only prime quality fish can be used. The main problem faced by the small fishermen is to preserve the tuna's prime quality. This is due to the fact that most of their fishing boats have no insulated fish holds and almost no facilities for the proper handling of fresh fish. In this study fish were preserved by chilled sea water (CSW). The results showed that using this technique, good quality fresh fish could be obtained, and the fish hold was efficient enough to give a saving in ice consumption.

1. INTRODUCTION

The geography of Indonesia makes the country well suited for both marine and inland fisheries. The potential source of fish is about 4,500,000 t (excluding the EEZ, potentially 2,100,000 t); out of this only one quarter has been exploited to date (Ilyas et al., 1988).

The production of the Indonesian fishery sector, however, still relies heavily on marine fisheries which contributes about 76%. The total production in 1987 was 2,669,800 t with an average increase of 5.5% per annum (DGF, 1988a). The bulk of the catch consists of pelagic as well as bottom fish from coastal waters mainly clupeids, carrangids, and scromboids.

The Indonesian fishing industry is mainly small scale or artisanal in nature. The fishing fleet consists mostly of small fishing boats of 5-10 t capacity, some have out-board motors and only a few have in-board motors. However, fishery products play an important role in earning foreign exchange. During the period January to September 1987 total exports of fishery products reached 97,554 t valued US$430 million. The main export commodities were frozen shrimp, frozen tuna, frozen frog legs and some cured products. In terms of volume and value, frozen shrimp was the largest export commodity followed by frozen tuna, the value of these two commodities are US$332 million and US$27.8 million respectively (DGF, 1988b).

2. DISCUSSION

2.1 Prospect of Fresh Tuna as a New Export Commodity

As mentioned previously during the last two decades frozen shrimp was the leading export commodity, but in recent years there is an increasing demand for fresh tuna for export to Japan. At present 200 t of fresh tuna per day is required by the Japanese market but only 12 t was supplied by Indonesia. Being in high demand, fresh tuna attracts a relatively higher price than frozen tuna. The current price of fresh tuna is US$3,300-4,000/t, while frozen tuna is only US$900-1,000/t (DGF, 1988b).

There is a large potential supply of tuna in Indonesian waters, especially in eastern zone (Maluku, Sulawesi and Irian Jaya), spreading out between West Sumatra, South Java and Bali (Figure 1). Estimated total potential of tuna is 80,000 t, but out of this only about 17% is being exploited. The species commonly caught are long tail tuna (Thunus tonggol), skipjack tuna (Katsuwonus pelamis), big eyed tuna (Thunus obesus), yellow fin tuna (Thunus albacares), albacore (Thunus alalunga) and frigate mackerel (Auxis thazard). Tuna can be caught throughout the year. However, the peak season is between September and December. Except for the fishing vessels of state owned and medium to large scale fishery enterprises, most of the tuna fleet consists of small vessels using long-line, gillnet, pole and line and purse seine as fishing gear.

To meet the rising demand of chilled tuna by the Japanese market, the Government of Indonesia has launched a program of expansion of the PIR (Small Scale Fishery Nucleus Enterprise) system, a scheme of cooperation between small scale fishery and state owned or larger fishery enterprise. This program is expected to boost the production of chilled tuna from 12 to 100 t per day.

A trial tuna fishing operation using the PIR system was initiated in 1986 in Sorong (Irian Jaya) as a pilot project. The result of this trial was very promising, producing 5,500 t of fish (4,400 t from the nucleus and 1,100 t from small scale fishermen). The system was designed not only to step up the production but also to improve the income of small scale fishermen. Since the program was launched, the monthly average income of the small scale fishermen increased from Rp30,000 to Rp140,000. The income of the owners of the fishing vessels also rose to an average of Rp1.6 million per month compared to only Rp500,000 per month before the implementation of the PIR system. Based on these results, the government is now encouraging private business firms to take part in the system. They were asked to develop separate PIR units in the various regions of Indonesia. Three areas having potential for the immediate application of the system are Central Sulawesi, North Sulawesi, and Ambon.

To support the programme, the Government also asked the trawler owners to participate in this program by modifying their underutilised trawlers into long-line vessels. The operation of trawlers was banned by the Government and there are now more than 2,000 trawlers of various capacities (from 10 to 100 t) that are not operated. By modifying trawlers for tuna fishing gear, it is hoped that tuna production will be increased.

In addition, the development of "rumpon" (fish lure system), a system used to localise the school of fish, will be included in this program. The "rumpon" consists of a bamboo or steel pontoon, while the attractor is made of leaves or old tyres, and the weights are cement, concrete blocks or anchors. These systems are placed in water of 1,500-2,000 m depth. "Rumpon" attract small pelagic fish in many ways:

- the attractor is an ideal place for some algae and plankton to grow
- as a hiding place for small pelagic fish from predators
- as a place for spawning
- as a place to hide from the light.

The school of small pelagics around the "rumpon" will in turn attract tuna and other large fish.

2.2 Handling of Chilled Tuna On-Board

To meet the quality specification for fresh fish for the Japanese market, fish should be handled in a rapid and proper way so as to retain the prime quality of the catch for as long as possible, and to reach the importing countries in not more than 12 days after fish landing. In Japan, fresh tuna is usually prepared as sashimi, which is consumed raw. Therefore, only fish of prime quality can be prepared into sashimi, and only fish of more than 30 kg is suitable.

To study the handling practice of tuna which is to be exported as chilled product, observations of handling on-board fishing vessels, at landing place, and of its preparation prior to transportation by air has been carried out. The fishing gear observed were on long-line vessels of 200 t capacity, and the fishing operation extended over 6-10 days. After being caught, the fish were laid on the deck which had been lined with wet gunny bags, and the tuna killed by spiking the fish using Taniguchi tool and piercing the brain through the small whitish mass located between the eyes. Bleeding was done by cutting the vein through the linea lateralis close to the lateral fin. Fish were then wrapped in a gunny bag and tied. There is still some controversy as to whether gutting and the removal of the gills should be done before wrapping in the gunny bag or after the fish is landed.

Before being chilled, it is necessary for the fish to be pre-cooled using iced water. Chilling on-board could be carried out either by refrigerated water or by crushed ice. Water used for pre-cooling and chilling was a mixture of fresh and sea water with the ratio of fresh water to sea water of 1:2 to 1:4.

At the landing place, the fish were removed from the fish hold using a crane, unwrapped and then delivered one by one using a palanquin-like carrier to the processing plant in a belly-up position, in such a way as to maintain a straight position and to avoid bruising or cuts. The fish were kept in tanks containing iced water. If the fish were not already gutted, gutting and removal of the gills would be performed rapidly and carefully. The quality inspection was carried out by observing the appearance, colour, and texture through a cut at the beginning of the caudal fin. After being weighed, the fish were packed in a carton lined first with a polyethylene film and then with foam 1-1.5 cm thick and finally with polyethylene film. Some polyethylene bags containing about 0.5-1 kg of crushed ice were put in the belly and gill cavities. Other ice bags were placed around the fish. A total of 5 kg of ice was used in each box. For a box of 170x40x40 cm in size, about 2 to 3 fish weighing 90-100 kg in total were packed. Finally, the packed fish were loaded into refrigerated trucks to be delivered to the airport.

From these observations it was noted that there was no difficulty in carrying out such a complicated handling method on a fishing vessel which has the facilities necessary for handling of fresh fish. But for small fishing vessels commonly used by small scale fishermen it is doubtful if such an operation can be carried out properly. To overcome this problem an alternative technique which can be adopted by small scale fishermen should be developed.

2.3 Study on the Use of Insulated Fish Hold in Handling of Pelagic Fish

The main problem in the handling of fresh fish, especially for pelagic fish, is the perishability of fish due to its biochemical properties which are commonly high in lipid content and susceptible to physical damage due to its delicate skin and texture. The situation becomes more complicated due to high ambient temperatures prevalent in tropical countries like Indonesia. Therefore handling should be carried out with extra care, otherwise spoilage will take place very rapidly.

To overcome these problems, a study on the use of insulated fish holds in handling of pelagic fish has been carried out using purse seine and gillnet boats. The insulated fish hold was designed to suit the capacity and construction of fishing vessels commonly used by small scale fishermen. The insulating materials were styrofoam and polyurethane, while fibreglass was used as lining material. The insulating effect of a prototype fish hold in a gillnet boat is shown in Table 1.

Fish were cooled by the chilled sea water (CSW) technique by putting the fish into the insulated fish hold containing sea water and crushed ice, and the temperature of fish was maintained at approximately $0^{\circ}C$.

From trials on handling of pelagic fish during fishing operations in the Bali Straits using a purse seiner, it was found that this technique was efficient in maintaining the prime quality of the catch. The fish could be used as a quality raw material for canning. Comparisons between different methods of chilling and traditional handling without ice, can be seen in Table 2.

There are several advantages of using CSW as a cooling medium; (1) the catch price is 2-3 times higher than those handled traditionally, (2) fishing period can be extended, (3) at the landing place the catch still can be kept for some time in the fish hold without any fear of spoilage, while waiting for a better auction price, (4) low maintenance cost of insulated fish hold. It seems that handling fish by CSW was suitable for the Indonesian fishing industry which is still in the artisanal stage. The technique is efficient in maintaining the quality of the catch, easy to operate and suitable for handling pelagic fish. In addition to these benefits, oxidation of lipids and physical damage can be minimised. Moreover, all necessary materials required for construction of insulated fish holds are available and relatively inexpensive.

The fishing operation trial using CSW gained a very good response from fishermen and fishing boat owners. At the moment more than 60 purse seine units and 60 gillnet units have been equipped with insulated fish holds. The development of CSW technique was followed by a sudden increase in the use of ice for on-board handling of the catch. There is also an increasing demand for materials and expertise required for fish hold construction.

2.4 The Possibility of Chilling Technique Application in Handling Tuna in Small Scale Fishing Vessel

The Government program aims to increase the export of tuna to Japan through the modification of underutilised trawlers for tuna fishing, especially around the "rumpon" or "payaos" (deep sea "rumpon") and it is worthwhile studying the use of different methods of chilling fish which can be applied to small scale fishing vessels. To modify the fishing vessels, it is necessary to design changes not only to suit the fishing gear, but also to construct a good and efficient insulated fish hold for handling tuna of size more than 30 kg.

The use of ice or CSW for pre-cooling and storage of tuna still needs intensive study, especially in relation to the size and volume of the catch, fishing period, design and construction of fish hold, stability of the vessel, handling and unloading techniques and the quality requirements of the importing countries.

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SEAWEED/FISH POLYCULTURE, A NEW APPROACH IN THAILAND

by

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ABSTRACT

It was found that in some coastal areas of the Andaman Sea, the cage culture of marine fish has become a well established industry and the agarophyte algae (or agar bearing seaweed) are appearing as fouling organisms attached on the net cages.

The BRU has set up a pilot farm for polyculturing such seaweeds together with marine fish in an open sea environment. It was found that the seaweed could be harvested from the fish cages every month with a production rate of 1 to 1.2 kg of dry seaweed per one standard size fish cage of 4x4x2 m.

The BRU has also developed a simplified method for agar extraction of the seaweed collected from the fish cages. This technology is suitable for transfer to the rural areas for local production of crude agar which can be used in local foods. In addition, the crude agar is also suitable for refining with more sophisticated techniques to produce high-grade agar for other applications.

1. INTRODUCTION

Thailand, a traditional user of food grade agar, has demonstrated a growing demand for agar in the food industry and for laboratory applications. In 1986, Thailand imported more than 250 t of agar valued at over US$4 million. It is anticipated that Thailand's agar demand will continue to increase at an average annual rate of 8-10%.

In order to reduce the country's dependency on agar imports, the Royal Thai Government started a vigorous program on seaweed production and processing under the Agricultural Technology Transfer (ATT) subproject in 1986. The major focus of the program is to stimulate private investment in the seaweed processing industry for the local manufacturing and marketing of agar and also to create employment for the coastal population. The Department of Fisheries (DOF) of the Ministry of Agriculture and Cooperatives cooperated with Kasetsart University in the development of seaweed production process. Meanwhile, seaweed processing was carried out by the Department of Chemistry, Faculty of Science at Srinakarinvirot University.

2. DISCUSSION

Under the ATT program, the Biopolymer Research Unit (BRU) has been established and equipped at the Department of Chemistry, Faculty of Science, Srinakarinvirot University (Bangkok). Its task is to work on the identification, analysis and process development of seaweed in Thailand.

During the ATT subproject, seaweed production was planned to be based on locally available raw materials. Along the more than 2,600 km coastline of Thailand, Gracilaria species are abundant. Agarophytes samples collected in a survey, were identified by Dr I. Abbott of the Department of Botany of the University of Hawaii. There were four Polycarvernosa and four Gracilaria species in the Thai samples. Some of the Gracilaria species were of spermatangial arrangement. Production was found to be rather seasonal along the coast of the Gulf of Thailand and less seasonal along the Andaman Sea coast. Farming of the seaweed was also attempted on the East Coast with discouraging results due to siltation caused by indiscriminate fishing practices in the Gulf of Thailand. Other potential areas for seaweed farming development are in the Songkhla Lake where the floating vertical net method of cultivation is practiced and in Pattanee Province where bottom long-line fishing as well as pond culture is practiced.

An unexpected discovery by the BRU staff in October 1987 opened up new possibilities in the supply of raw material for agar extraction. It was found that thick Gracilaria mats were growing, attached to marine fish cages set up along many canals which open into the Andaman Sea. The waste

of the cultured fish serve as fertiliser for the seaweed while the oxygen produced during the phytosynthesis by the Gracilaria keeps the fish healthy.

The BRU has since set up a pilot farm for polyculturing Gracilaria together with fish in an open sea environment. It was found that the seaweed could be harvested from the fish cages every month with a production of 1 to 1.2 kg of dry seaweed per one standard fish cage of 4x4x2 m.

It is estimated that there are more than 200,000 marine fish cages in Thailand. The seaweed growing on them are presently considered as pestiferous fouling organisms and are discarded during regular cleaning operations. When properly utilised, this resource alone would be enough to supply the raw material requirement of a commercial agar producing plant. At the same time considerable supplementary employment and income could be generated in the coastal areas by the collection and processing of the agarophytes from the existing cages and the expansion of this fish/seaweed polyculture.

The processing team of the BRU collected seaweed samples for agar extraction and analysis. It was found that with the traditional extraction method of the commercial factories, the seaweed samples gave an agar with a gel strength of only 300-400 $g.cm^{-2}$. When the newly developed technique of post-extraction alkali treatment was applied to the same samples, the get strength of the agar improved to 800 to 1,000 $g.cm^{-2}$. The agar samples prepared by the BRU team are considered to be high grade agar. Other physical and chemical properties of agar samples produced from seaweed collected in different parts of Thailand are shown in the following Table.

Table 1

Physical and chemical properties of several agar samples

	SU 0014 Kuntung Trung Province	HAI-0020 Lam Hin Trat Province	HAI-0029 Ko Yaw Songkhla Province
Gel strength ($g.cm^{-2}$ at 1.5%)	863	1,000	947
Gelling temperature (^{o}C at 1.5)	36	36.5	37.5
Melting temperature (^{o}C at 1.5%)	93	86	96
Sulfate content (%)	0.7	0.08	0.94
3,6-Anhydrogalactose (%)	-	40.4	34.15
Ash content (%)	-	0.7	-
Moisture content (%)	16.5	16.9	16.8
Protein content (%)	-	-	0.91
Yield (% of clean dry seaweed)	29.5	22.3	26.7

The agar samples were also analysed to detect heavy metals (lead, arsenic, mercury and others) by an atomic absorption spectrophotometer, but none was found. The samples of HAI 0020 and 0029 were identified as Polycarvernosa fastigiata and P. fisheri, respectively. The sample of SU 0014 was from the BRU pilot farm and was identified as Gracilaria sp.

The Biopolymer Research Unit which is now serving as a national centre for phycocolloid identification, analysis and research, is able to offer its services for the benefit of tropical countries of Asia and the Pacific. Samples of agarophytes may be sent to the BRU in order to determine its agar quality and quantity and to propose a suitable process for its extraction. Information exchange and other ways of cooperation may also be offered to institutions interested in developing their countries phycocolloid production potentials.

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